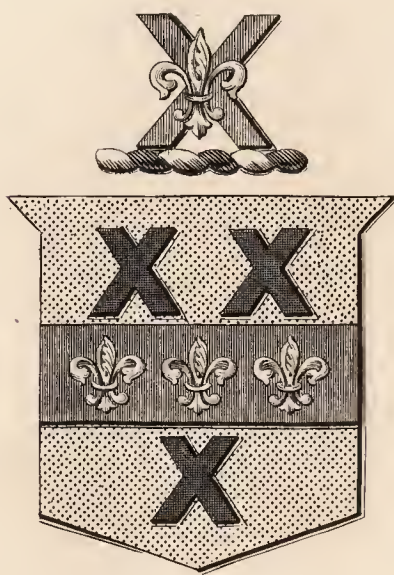


THE
COLLOIDAL STATE
IN ITS MEDICAL AND
PHYSIOLOGICAL ASPECTS

SIR WILLIAM BAYLISS

OXFORD MEDICAL
PUBLICATIONS




E. BARCLAY-SMITH, M.D.



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THE COLLOIDAL STATE
IN ITS MEDICAL AND
PHYSIOLOGICAL ASPECTS

BY

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P R E F A C E

THIS monograph is to be regarded as an attempt to give a short account of the properties of colloids, so far as of interest in connection with the phenomena occurring in living beings.

In many cases the purely chemical properties of substances in the colloidal state are similar to those of the same substance in true solution. Hence it is evident that less consideration is needed of chemical properties in such cases, except where they are responsible indirectly for the special behaviour of the same substance in the colloidal state.

Although the characteristics of the colloidal state are mainly physical, or rather physico-chemical, the chemical aspects of the problems are not neglected. Thus the book is neither one on the physics nor on the chemistry of colloids, but tries to include both.

In point of fact, the boundary between these two branches of science is rapidly becoming obliterated. It looks as if, before long, we shall not be contented with a statement that such and such a particular phenomenon is chemical, but demand an explanation in terms of the physics of the atom.

W. M. BAYLISS.

UNIVERSITY COLLEGE, LONDON,
October 1922.

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THE COLLOIDAL STATE IN ITS MEDICAL AND PHYSIOLOGICAL ASPECTS

CHAPTER I

THE NATURE OF THE COLLOIDAL STATE

General Nature

ALTHOUGH the nature of the colloidal state in its typical form is easy to recognize, namely, as a suspension of separate solid particles or liquid globules in a continuous medium of a different kind, it is sometimes a matter of difficulty to be satisfied when the particles become very minute. Thus, the particles of arsenious sulphide usually consist of several thousand molecules, and it is natural to inquire how many molecules are necessary to possess the properties required of a colloid when they are aggregated into a particle. Suppose that we were to divide a little lump of gold into smaller and smaller pieces. At first these pieces would sink rapidly in water and this would be obviously a coarsely heterogeneous system. If we could proceed far enough, we should at last arrive at the separate molecules, and these would then be said to be in true solution. But, at a certain intermediate stage, the particles, although consisting of many molecules, would not

fall as a deposit, but remain in permanent suspension. This stage is what is known as the colloidal state (see Fig. 1).

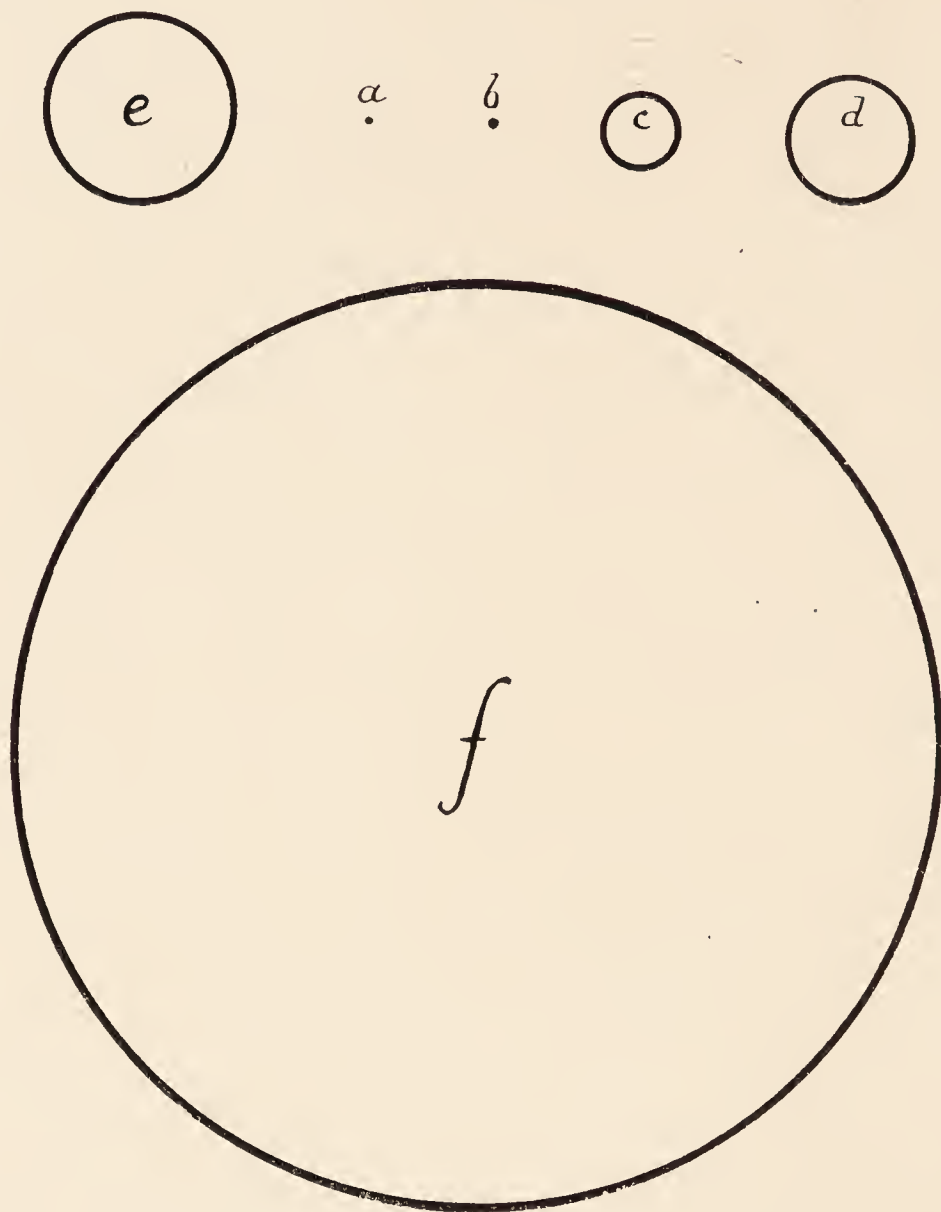


FIG. 1.—Relative Dimensions of Molecules and Colloidal Particles. Magnified one million times.—After Zsigmondy.

- a.* Molecule of alcohol.
- b.* Molecule of chloroform ($0.8 \mu.\mu$).
- c.d.e.* Particles in colloidal gold solutions (6 to $15 \mu.\mu$).
- f.* Particles which deposit ($75 \mu.\mu$).

Criteria of the State

A rough practical test is that used by Graham, who was the first to describe colloids and to give them the name. It should be remembered, however, that Faraday had already made what was in fact a colloidal

solution of gold, and recognized its nature as a suspension of minute particles of solid metallic gold. Graham used as a test the capacity of passing through parchment paper. If the particles were too large to pass through the pores of this paper, they were said to belong to a colloid. It will be clear that this is rather an arbitrary limit. If it is satisfied by a solution which remains without deposit on standing, there is no doubt that we have a colloidal solution. But it is not so easy to say whether certain particles, although composed of many small molecules, might not be sufficiently small to pass through parchment paper; or, on the other hand, whether there may not be single molecules or ions of dimensions so large as to be unable to pass through. Proteins are believed by Loeb to be in such a case, but we shall see reason later to doubt the correctness of this view, since there appear to be units of smaller size than those in the usual solutions of proteins.

The use of parchment paper or collodion is a valuable means of separating colloids from diffusible salts present in their solutions. The method is known as *dialysis* (Fig. 2). Frequent change of the water on the opposite side of the membrane removes the salts rapidly.

The real characteristic of the colloidal state, in contrast to the molecular or ionic one of true solution, is that the particles are large enough to possess the properties of surface. What these are will be seen in

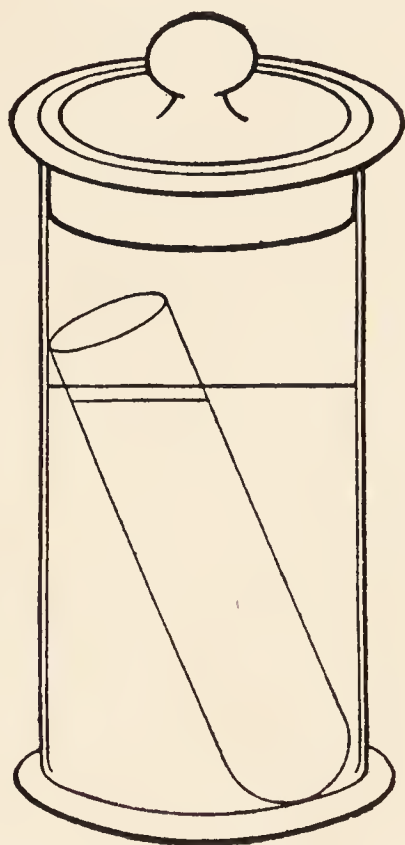


FIG. 2.—A Form of Dialyser.
—Ostwald-Eischer.

the next chapter. But the difficulty arises as to how many atoms or molecules are necessary for this purpose. The work of the Braggs has shown how the surface of a crystal is made up of a definite arrangement of the constituent atoms of the compound. Thus, in sodium chloride, each sodium atom is surrounded by six chlorine atoms, or five if on the surface, none of which belongs solely to that sodium atom (Fig. 3). In the

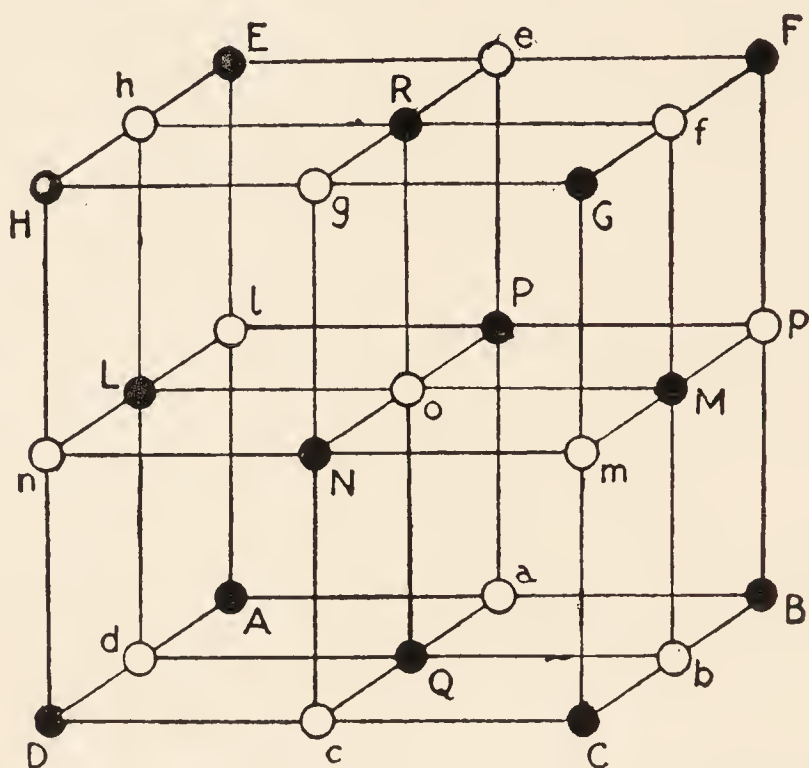


FIG. 3.—Bragg's Model of Sodium Chloride Crystal.—Bragg's *X-Rays and Crystal Structure*.

Black—Na atoms.

White—Cl atoms.

crystal, there is no molecule of NaCl. Whether we call the forces which unite these various atoms in the crystal chemical or physical seems to be a matter of taste. If we have a very large molecule, such as those of some organic compounds, it does not appear impossible that there might be sufficient atoms to have the properties of surface. But what evidence we have points to the non-existence of these large molecules in the separate state, except, perhaps, in very dilute solution; they readily form aggregates, whether in

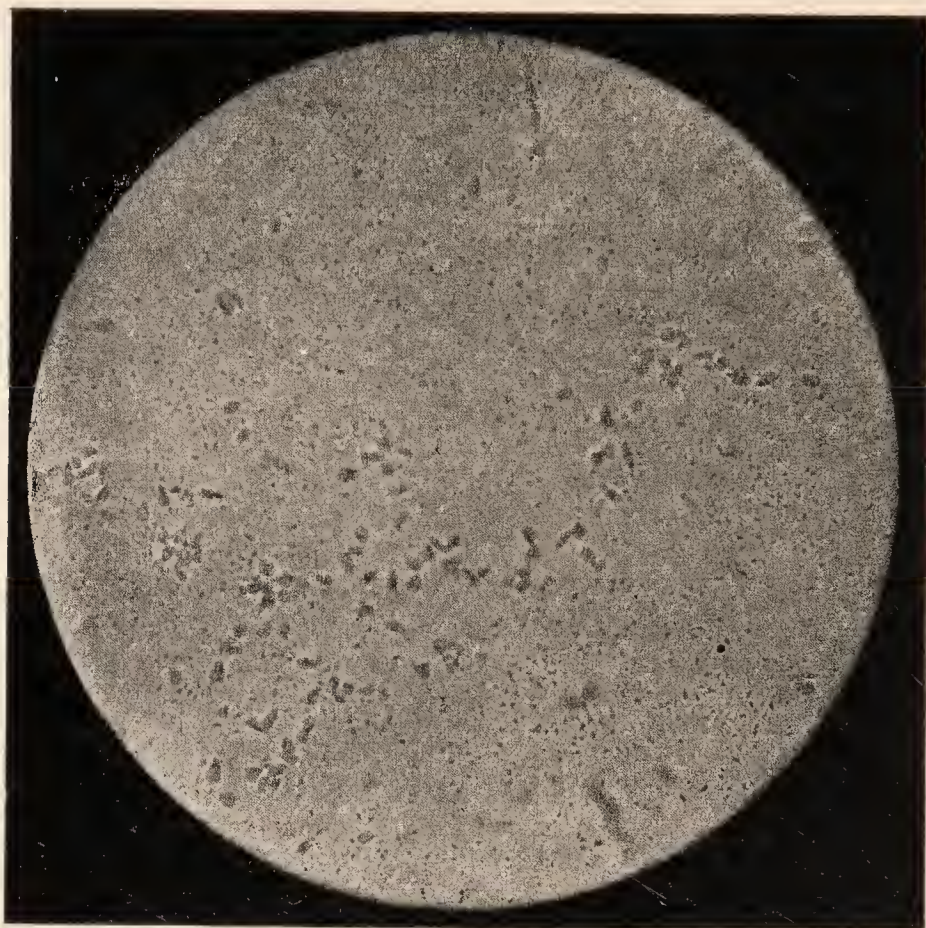
the undissociated state or as ions. The evidence will be found chiefly in the osmotic pressure of their solutions and is dealt with in Chapter IV. below.

Colloids and Crystalloids

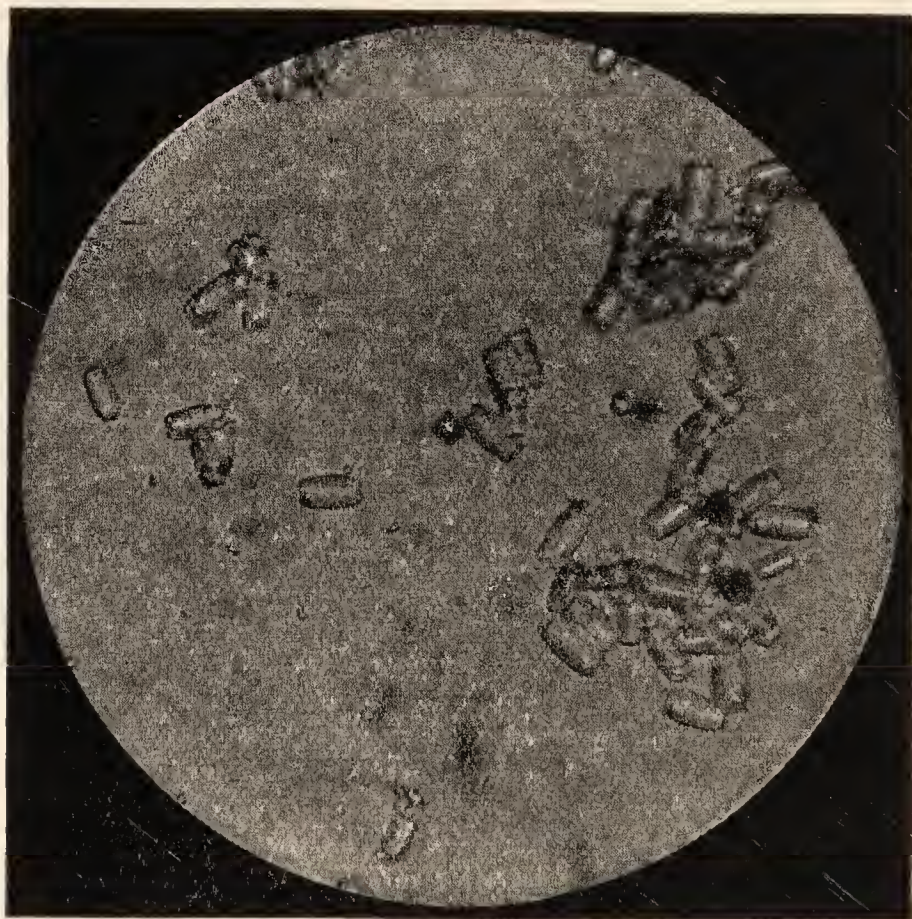
It will be remembered that Graham made a distinction between colloids and crystalloids. It is true that many of the former, especially the organic ones, are difficult to crystallize, but there is no difference in principle between the cases. When barium sulphate is first precipitated, the constituent atoms of the particles have not had time to arrange themselves into the geometrical pattern of the crystal. But this takes place in course of time (Fig 4). It is really a question as to small particles which possess surface, and these may or may not be crystalline. Indeed, we may have a colloidal solution of minute crystals of such a typical "crystalloid" as sodium chloride, if suspended in saturated solution. Such a system would, however, not be permanent, since the larger crystals grow at the expense of the smaller ones and ultimately become large enough to deposit.

Surface and Mass

The question will naturally be asked, why do not large particles which deposit deserve the name of colloids, since they have surfaces? In point of fact, they do possess the essential properties of colloids, but the proportion of surface to mass is so small that its properties are scarcely to be detected. This may be realized by a simple calculation. A sphere of gold of one millimetre in radius, if divided into particles of the dimensions of those in a colloidal solution, has its surface increased a million times.



A



B

FIG. 4.—Barium Sulphate.—Von Weimarn. (“Kolloid-Zeitsch.”)

A. One hour after precipitation.

B. Six months later.

The fact that this possession of surface confers additional properties of a different nature from the purely chemical ones may be seen from the observations of Graham, that a trace of carbon dioxide causes gelation of a colloidal solution of silicic acid, and that a small amount of sodium sulphate completely precipitates ferric hydroxide. This colloidal ferric hydroxide also presents the apparently contradictory properties of being precipitated both by acids and alkalies.

Heterogeneous Systems

It will now be understood that colloidal solutions are special cases of heterogeneous systems. They have two parts, which do not mix with one another. Willard Gibbs suggested the name "phase" for each component of such systems. In ordinary cases, the phases are large enough to be more or less easily separated from one another by some mechanical means. In colloidal solutions, the dispersed phase of the particles is in extremely minute subdivision and has special properties on this account, as will be seen in the next chapter. For this reason, they are sometimes called "micro-heterogeneous."

That phase of a colloidal solution which consists of the separate particles is usually called the "dispersed phase," while the medium in which they are suspended is the "continuous phase," for obvious reasons. The dispersed phase is also called the "internal" phase, because it is completely surrounded by the continuous "external" phase.

It is of interest to consider the evidence that colloidal solutions are heterogeneous. The fact that they do not diffuse through membranes which have pores of measurable dimensions is to some extent a proof of this. But the clearest demonstration is afforded by the ultra-microscope.

The Ultra-microscope

This instrument is a development of Faraday's observations on colloidal gold. He found that a bright beam of light sent through what appeared in ordinary light to be a clear solution, showed as a yellow track when looked at from the side. He rightly

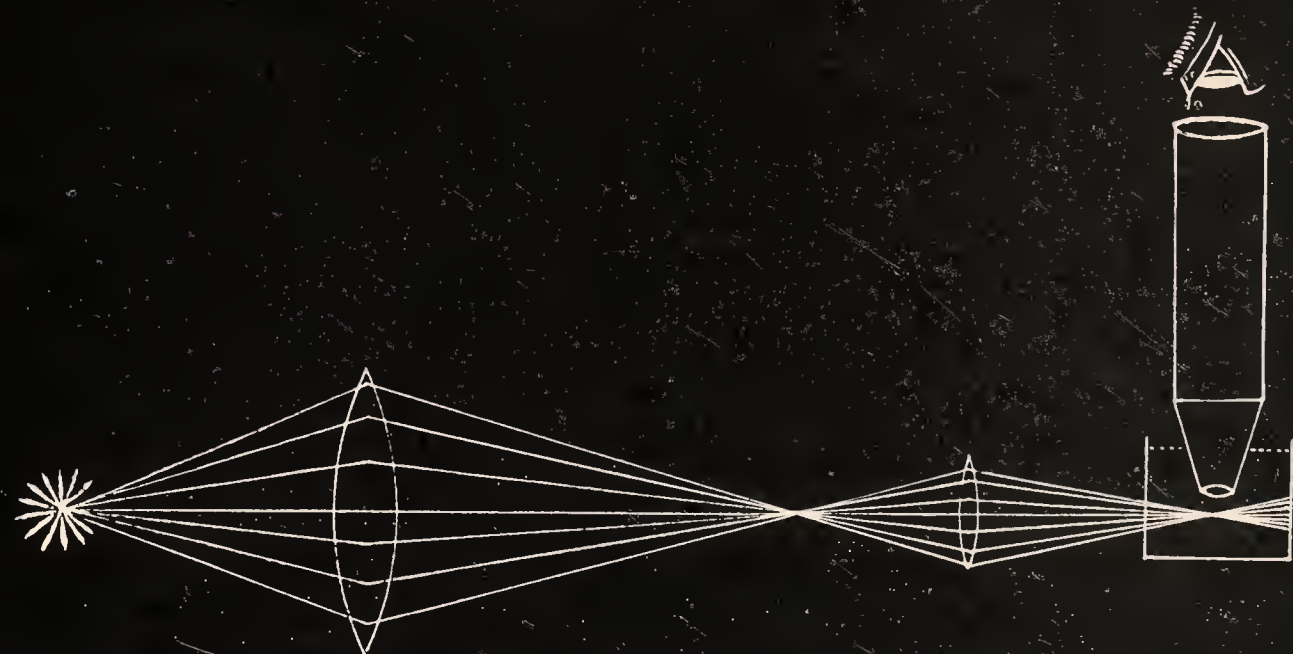


FIG. 5.—Diagram of the Course of the Rays of Light in the Ultra-microscope.—Bayliss, *Principles of General Physiology*. Longmans.

interpreted this as being due to light reflected, or rather diffracted, from solid particles of metallic gold. Tyndall, at a later date, noticed that the light was often polarized, which means that in such cases the particles have dimensions similar to the wave length of the illuminating beam. Siedentopf and Zsigmondy, still more recently, arranged a microscope to observe the beam, and by this means were able to see separate

particles by means of their diffraction images (see Fig. 5). The same thing is done by the various methods of dark-ground illumination, in which a brilliant illuminating cone of light is so directed as to pass obliquely through the preparation, while all direct light is prevented from reaching the eye of the observer by means of a central opaque disc in the condenser (Fig. 6).

While this ultra-microscopic method is a valuable one for the investigation of colloidal solutions, it has its limitations. Thus, the more minute the particles, the more brilliant must the illumination be, if separate particles are to be seen. Moreover, if the refractive index of the particles approaches that of the dispersion medium, the light diffracted will be very faint.

A further source of error may arise if quantitative conclusions as to the amount of colloidal matter present are drawn. The method, in fact, is a very sensitive one, and a solution containing very little of its contents in the colloidal state may show a large number of particles, if examined undiluted. It must be examined in a state of dilution comparable with that of a solution known to have all of the particular compound present in the colloidal state. Certain commercial preparations, although showing particles with the ultra-microscope, have been found to contain nearly the whole of the matter present in true solution and diffusible through parchment paper.

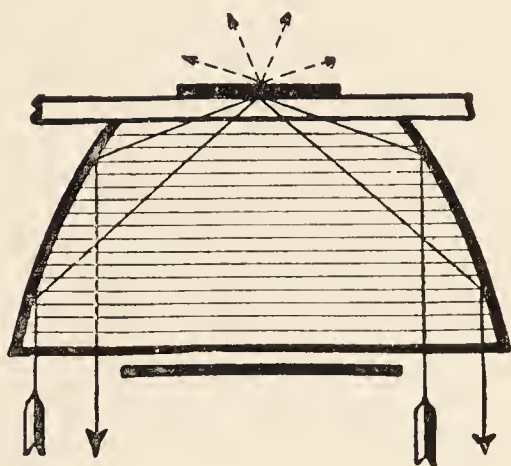


FIG. 6.—Condenser for Dark-ground Illumination.

Solubility

The degree of solubility in the molecular or ionized state plays a large part in the ease of production of colloidal solutions. Thus, while silver nitrate forms a true solution in high concentration, and very special conditions are necessary to obtain it in the colloidal form, silver chloride is readily obtained in colloidal particles, because its true solubility is very low. But delicate methods, such as that of the concentration battery, show that it is not absolutely insoluble. The same statement applies to many other substances, if not to all. This being so, it is clear that the particles in a colloidal solution, or "sol," of such substances are suspended, not in pure water, but in an extremely dilute true solution. This was pointed out by Quincke and by Hardy. Recent observations by Cohn, by Schryver and by S. C. Bradford show that even in the case of proteins there is a real true solution as molecules, but of an extremely low degree, 70 to 110 milligrams per litre. If this be the case, it is clear that the ordinary solutions of these substances do not consist of single molecules, as is sometimes supposed, but of colloidal aggregates, a conclusion to which osmotic pressure measurements also lead, as remarked above. Further, the jelly of gelatin in dilute alcohol, investigated by Hardy, does not consist of a solid phase containing a small concentration of solvent, with a dilute true solution in its meshes, but the latter is rather a colloidal sol, and the system is one of a solid, containing little solvent, in equilibrium with a suspension of particles of the same material in an extremely dilute true solution, and might with justice be regarded as a system of three phases—the fixed structure, the colloidal solution and the true solution.

The commercial colloidal solution of iodine is of

interest in this connection. Iodine is soluble in water to a certain degree (1 part in 5500). Hence, so far as such preparations contain colloidal iodine, this must be present suspended in a true saturated solution. The question naturally arises in such cases as to how far their activity is due to the iodine in true solution, as we shall see later. Of course, the so-called colloidal solutions of iodine in oil contain a larger amount in true solution than the hydrosols do.

Stability

We have seen that what distinguishes a colloidal solution from a suspension of coarse particles, which rapidly sink to the bottom, is the permanent suspension of the much smaller particles of the former, and it is natural to ask why do very small particles remain suspended?

The answer is to be found in the possession of Brownian movement, aided by the electric charge which most colloids possess. Although the fact that the particles are in incessant movement in all kinds of directions is most clearly seen in the ultra-microscope, the larger particles visible under ordinary methods of microscopic observation also show the same movement, as was discovered by the botanist, Robert Brown, in 1828. Until the work of Perrin in 1908 the cause of this phenomenon was obscure, although Ramsay had suggested the true explanation in 1879. Various hypotheses had previously been ruled out, and Perrin was able to show by a series of ingenious experiments that it is a visible manifestation of the molecular movements of gases and liquids postulated by the kinetic theory. Consider minute particles suspended in water. The molecules of water are in perpetual movement in all directions and, owing to their possession of

mass, they have also kinetic energy. The solid particles will therefore be continually hit by these molecules in all sorts of directions, first on one side, then on another. Thus, as they tend to fall, they will before long receive a blow from underneath and sent upwards again. The reason why it is only very small particles that behave thus is that the molecules of water are small in comparison with the particles, so that these latter are hit in various directions at the same time. Being small, however, there is not an equal balance on opposite sides, and the net result is a movement in some direction. Large particles, on the contrary, are hit by so many water molecules in all directions at the same time that the resultant kinetic energy imparted is insufficient to counteract the weight of the particle.

Suppose next that in their random movement two of these colloidal particles are projected so as to hit one another. Such collisions would in some cases result in the particles sticking together and ultimately becoming large enough to fall as a precipitate. But if they possess electric charges of the same sign, they repel one another and are thus prevented from collision, as can be seen under the ultra-microscope.

A further aid to the permanence of colloidal solutions is the viscosity of the suspension medium. The rate at which a particle falls is lessened by increased viscosity, so that in the case of particles near the limit, it is clear that there is more chance of their being hit in the right direction before they have fallen too far. Bismuth carbonate is prevented from rapid deposition by the addition of gum arabic.

Another factor is the specific gravity of the particles. If they had the same specific gravity as the medium, they would not fall in any case. But this is practically never met with. They are usually heavier, occasion-

ally lighter, than the medium of dispersion. A similar consideration to that of the previous paragraph as regards rate of fall applies to this factor.

Suspensoids and Emulsoids

There is an important distinction between two classes of colloids in respect of the composition of the particles. In such cases as those of gold, arsenious sulphide and so on, the particles consist of pure solid. But in most of those of physiological interest, such as the proteins, they contain more or less water as a constituent. Thus, there is less difference between their composition and that of the solution in which they are suspended than in the former case. At the mutual boundary surface, there is a less violent change of properties than when water is present in one phase only. Colloidal solutions typified by that of gold are called *suspensoids*, while those whose dispersed phase contains water belong to the *emulsoids*. The latter class is sometimes defined as that in which both phases are liquid, such as an emulsion of oil in water. But as regards its essential properties, when the dispersed liquid does not contain water, a system of this kind behaves more like a suspensoid. It seems that it would be better to confine the name emulsoid to those cases where the internal phase contains more or less water, although it may sometimes approximate more to the character of a solid.

Imbibition

A property of emulsoids which plays an important part in the mechanics of cell processes is that to which Hatschek has especially called attention. The amount of water contained in the particles can be altered by the presence of salts and other soluble substances.

When we remember that the direction in which an enzyme works, whether hydrolysing, as glycogen to sugar, or synthesizing, as when glycogen is formed from sugar, depends on the free water present, we obtain some light on these obscure intra-cellular processes. It may be mentioned that the water taken up is sometimes called that of *imbibition*, more especially when the colloidal system as a whole partakes of the nature of a solid, as in the jelly formed from gelatin. The mechanism is not yet fully understood and will be referred to again later.

Varieties of Systems

Colloidal systems are not exclusively confined to

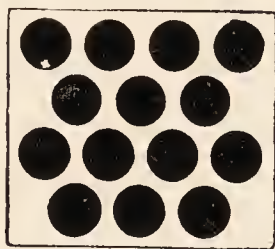
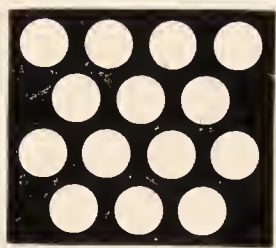


FIG. 7.—Diagram of Reversal of Phases.

those of solid and liquid, or liquid and liquid, while in the latter case either liquid may be the internal phase (Fig. 7 illustrates the difference between cream and butter).

The following table gives some interesting examples of various kinds of colloidal systems.

INTERNAL OR DISPERSED PHASE.	EXTERNAL OR CONTINUOUS PHASE.	EXAMPLE.
Gas.	Liquid.	Foam.
Liquid.	Gas.	Fog.
"	Another immiscible liquid.	Emulsion or emulsoid milk.
"	Solid.	Certain forms of jelly.
Solid.	Gas.	Tobacco smoke.
"	Liquid.	Ordinary colloidal solutions, as gold, etc. (suspensoids).
"	Another solid.	Ruby glass.

CHAPTER II

INTERFACIAL PHENOMENA

Nature of Interface

WE have seen that the special properties of the colloidal state are due to the large development of surface, and our next problem is to inquire into what are the phenomena particularly associated with surfaces.

When two things which do not mix with one another, such as a liquid and a solid immersed in it, that is, two phases are in contact, it is clear that the atoms or molecules forming that layer of each phase which is in contact with the other are exposed to forces different from those to which the molecules in the interior of each phase are exposed. The former are exposed on one side only to the action of molecules similar to themselves, on the other side to the molecules of the other phase. Thus the surface, or interface, of contact is composed of two layers, as it were, one belonging to each phase.

The attractive forces which hold molecules together are spoken of as "cohesion" when between similar molecules, or "adhesion" when between different molecules. But these are essentially similar, although different in degree. Thus, the molecules at an interface are attracted more powerfully in the direction of the phase to which they belong, than in the opposite direction.

Surface Tension

In the case of liquids, as was shown by Thos. Young and by Laplace, this attraction between the constituent molecules gives rise to a state of tension at the free surface. For the sake of simplicity, let us consider the surface of a liquid in contact with a gas. Since the molecules of a gas are sufficiently distant from one another for the attractive forces of cohesion to be absent, we have only to take into account the surface layer of the liquid itself, the second component of the interface being non-existent. Now, while the molecules in the interior of the liquid are exposed to equal forces on all sides, those at the surface are only attracted in the direction towards the interior of the liquid, the forces in the opposite direction not being present. Since the surface cannot move inwards, owing to the incompressibility of the liquid, the result is that the pull inwards shows itself as a state of tension, such that the surface takes up the smallest area possible.

The existence of such a state of tension is readily shown with soap films. Perhaps the most instructive experiment of this kind is to take an ordinary glass funnel, close the end of the stem with the finger, and form a film over the wide end by immersing it in a soap solution and withdrawing it. If the finger is then removed, the film will be seen to move upwards to the narrowest part of the funnel, where it has the smallest area, driving out the air above it in the process. We see also that surface tension, or rather the surface energy due to its existence over an area, can do work by raising the weight of the film of soap solution. Energy is lost in the diminution of the area of the film.

Now, while surface tension in this form can only be imagined in the case of the mobile molecules of a liquid, various phenomena which occur at the contact

between solids and gases show that forces of an analogous kind are present in the surfaces of solids. It is perhaps better to speak of them simply as due to cohesion, as A. P. Matthews prefers to do.

Suppose next that the surface possessing tension is curved, as that of a soap bubble. A little consideration will make it clear that the tension directed along the surface will have a component towards the concave side of the surface, and that this component will be the greater the more curved the surface is. Thus the pressure inside a small soap bubble is greater than that in a large one. With such curvatures as those of particles of the dimensions of the colloidal state, the pressure produced in the interior must attain a great magnitude. If the pressure inside droplets of water of such dimensions in air be calculated, it will be found to amount to some 150 atmospheres, and seems likely to have effects that need to be taken into consideration. It is remarkable that Sir Wm. Bragg has been unable to obtain evidence that these pressures have any effect in reducing the distance of the atoms from one another in particles of gold.

It will be remembered that Willard Gibbs put forward a law controlling equilibrium in heterogeneous systems, known as the "phase rule." A description of this rule is beyond the scope of the present monograph, but we may say that it enables the behaviour of coarsely heterogeneous systems to be foreseen. We are not yet able to state whether it applies to the minutely subdivided phase of micro-heterogeneous systems, as colloidal solutions are. Gibbs expressly considered only changes in pressure, temperature and volume, and it has been shown in the previous paragraph that surface tension must be taken into account in the case of colloids. Perhaps also the Brownian movement may in some way affect the

application of the law. Thus a modification in the phase rule may be necessary, but there is no doubt that if we were able to apply it with confidence, it would be a valuable help in the interpretation of the properties of colloids.

Electric Charge

It is found, as a matter of experiment, that the particles of colloidal solutions possess in most cases an electric charge, either of positive or negative sign. By appropriate means, to be discussed later, they can be deprived of their charges, but are then more or less unstable and tend to aggregate together and precipitate.

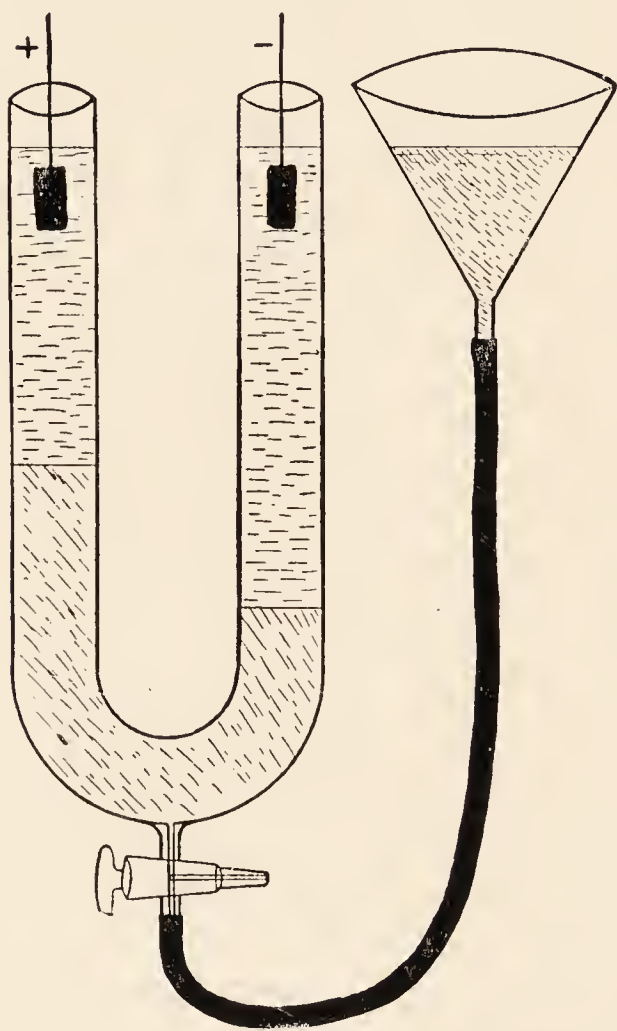


FIG. 8.—Apparatus for examining the Charge on Colloidal Particles. The upper part of each limb is filled with water, the colloidal solution run in from below.

The electrical state of the suspended particles can be ascertained, and to some extent measured, by placing in the solution two electrodes, connected respectively with the positive and negative terminals of a source of fairly high potential, such as the direct current lighting mains. The particles, if charged, are attracted to the pole of opposite sign ; or, if devoid of charge, do not move (Fig. 8). The magnitude of the charge is indicated by the rate of movement.

It is necessary to remember that the presence of a charge on a surface implies that of an equal and opposite charge in the immediate neighbourhood (Helmholtz double layer). If these charges were fixed in position, the system of particles, plus the other coat of the double layer, would have no externally effective charge and would remain motionless in the electric field. One must therefore suppose that there is a certain slip or "give" between the components of the double layer, as the particles move through the liquid. The charge on that part of the layer composed of molecules of the suspension medium must be transferred to contiguous molecules one after the other, the colloid particle being, as it were, handed on (see Lewis, *System of Physical Chemistry*, vol. i. p. 335).

As Faraday showed, an electric charge is localized on the surface of a body. We have to consider the possible ways in which such a charge may be given.

Adsorption of Ions

Solutions which conduct electricity do so, as is well known, by the presence therein of electrically charged "ions," positive or negative, derived from the dissociation of substances dissolved in them. These ions convey varying numbers of unit charges, "electrons," and deposit them at one or other electrode, themselves becoming neutral and deposited on the electrode.

If, therefore, colloidal particles, or indeed surfaces of any kind, are immersed in a solution which contains ions, and if there were any means by which ions of one or other sign could be deposited on the surface, it is clear that these charged ions would give to the surface a charge of corresponding sign.

Such a process is known as "adsorption," and we must first devote some attention to it in its general

aspect, on account of its importance in the behaviour of colloids.

Adsorption in General

The reader may be reminded, to begin with, of a fact of universal experience, namely, that free energy, or that which can be used for work, always takes the opportunity of losing its freedom and becoming, as it is called, "bound," usually by change to heat. Why this is so cannot be explained, but we know that any process in which it is possible for free energy to decrease will take place. A prediction based on this belief is never known to fail. It will be recognized as the second law of energetics or thermodynamics, in one of its forms.

Now the surface tension at any interface confers free energy there. We saw that this surface energy raises a soap film against gravity. It was pointed out by Willard Gibbs that if the deposition of any constituent out of a solution on to a surface immersed in it has the power of lowering the surface energy, it will occur. He also put forward a mathematical expression giving the degree of adsorption, in terms of the amount deposited in relation to the lowering of surface tension. It is to be remembered that the Gibbs' equation only takes account of surface energy resulting from mechanical tension, and that the general consideration applies to surface energy of other kinds, electrical or even chemical.

Like all thermodynamic reasoning, the conclusion that adsorption must occur under certain conditions does not pretend to explain the way in which it is brought about. Although deductions from the laws of energetics are always cogent, it is natural to demand further information as to how the phenomena are produced.

Now, in the first place, although it may happen that chemical combination between the material adsorbed and constituents of the adsorbing surface does sometimes occur, there are many facts that require some other explanation, since we find adsorption taking place without chemical combination. When this combination does occur, it is often found to be a secondary process. Thus, the complex coloured acid, which, combined with sodium, forms the dye, congo-red, can be obtained in the form of a deep blue colloidal solution. As an acid, this is capable of combining with bases, amongst others with aluminium hydroxide. Suppose that this base, in very fine powder or as colloidal solution, be mixed with the blue congo-acid, as we may call it, it is found that the particles are dyed blue by adsorption of the dye-acid. Now, the real salts are red, not blue, and if the preparation is left at room temperature, a slow chemical reaction takes place, more rapidly if warmed, with production of the red aluminium salt. The blue "compound" is clearly only a close apposition of the acid and base, not a chemical combination.

Again, charcoal is well known to adsorb large volumes of many gases, and was used for this purpose in the masks for protection against poison gas in the War. Chlorine is one of these gases, and it has been pointed out by Bancroft that if the taking up of this gas were due to chemical combination, it ought not to be so difficult as it is to obtain such compounds as carbon tetrachloride by direct combination. On the other hand, it has been suggested by Freundlich that the slow secondary disappearance of iodine from a solution in presence of charcoal, as observed by O. C. M. Davis, may be due to chemical combination, rather than to slow diffusion into the interior of the solid carbon.

Langmuir's theory of adsorption has been much

discussed in recent times. In a certain sense, or in certain cases, it may be called a chemical view ; but, as will be seen, the designation must be understood in a somewhat peculiar or special way. Taking first the adsorption of anions or cations by a crystal surface, say that of SO_4' or K' by sodium chloride, the process is regarded as the combination of such ions with potentially free valencies of the sodium or chlorine atoms in the surface lattice of the crystal. The sodium atoms in the interior are in union with other sodium and chlorine atoms on all sides. At the surface, this is not the case on the side facing the exterior. If we may look upon the forces uniting the atoms of a crystal to each other on all sides as chemical, then we may call this particular form of adsorption as being also chemical. But it seems rather a matter for personal preference. At all events, it will be clear that adsorption on this view can only be in a layer of one molecule in thickness. This is no doubt frequently the case ; but there are also cases in which it is difficult to account for the total quantity adsorbed on this basis.

Suppose that the compound adsorbed is one containing special groups which have an "affinity" for the material of the adsorbing surface, while others have more affinity for the liquid in which the surface is immersed, it is reasonable to hold that the molecules adsorbed will be orientated in such a way that the groups with affinity say for water, as the carboxyl groups of acids, will project outwards into the water, while the hydrocarbon part of the molecule is attached to the surface. If or when such an orientation occurs, it will be obvious that if two substances capable of reaction with each other are adsorbed at the same time, as by enzymes, the facility of reaction will depend on the way in which the interacting groups are arranged with respect to each other.

But here there seems to be some difficulty in Langmuir's views. Suppose the material of the adsorbing surface to be amorphous and inert, such as charcoal. It is difficult to accept the view of chemical combination in such cases, and indeed some observers find no evidence for it. While it is very likely that, *after adsorption*, the molecules become orientated by the affinity of certain groups for water, etc., no satisfactory hypothesis has yet been put forward to explain why they are first of all accumulated in greater concentration on the surface of the adsorbent than in the body of the solution.

It will probably have struck the reader that in extending the conception of chemical combination to adsorption, whose characteristic is that the chemical properties of the substance are unaltered, some violence seems to have been done to what used to be regarded as the great criterion of chemical combination in contrast to mechanical admixture, namely, the change of chemical properties. Such typical cases as mercury and iodine forming mercuric iodide, or oxygen and hydrogen forming water may be called to mind. It seems to the writer that the advocates of this wide extension of chemical combination are in danger of losing sight of the essential nature of the process. But, of course, it may be said that there are all degrees of transition between chemical and physical phenomena, and it is no doubt true that more and more progress is being made in the explanation of chemical phenomena in terms of the physics of the atom. Perhaps this is to be looked upon as a new science uniting chemistry and physics, forming the borderland between them.

A few words must now be said as to the mathematical aspect of adsorption. In the first place, its main characteristic is the continuous nature of the

curves which represent its magnitude in relation to the concentration of the solution from which the adsorption takes place. The temperature being constant, the curves referred to are known as "adsorption isotherms." While chemical combination occurs only in separate steps, as it were, to every concentration of the adsorbed substance there corresponds a definite amount of adsorbed material. If we call the result an "adsorption-compound," the above fact may be expressed as the possibility of existence of all kinds of proportion between its constituents.

As a first approximation, it is found that the proportion of the amount adsorbed to the concentration present follows a formula in which the former varies as a power of the latter and that this power is usually not a whole number. As a rule, the degree of adsorption becomes less for a given increase as the concentration rises. The curve is thus one of the family of parabolas and could be prolonged indefinitely. In point of fact, however, it appears that the complete process is not so simple as this. Above a particular concentration it is found that no more adsorption takes place with increased concentration. The surface is saturated. In some cases, however, it appears that saturation has been supposed to have been reached when what really happened was that above a certain concentration, solvent and solute were being taken up at the same rate. On Langmuir's view, it is easy to see that when the whole surface is covered with a layer one molecule thick, the process would be at an end. And whatever the active forces may be, their sphere of action must come to a limit at a comparatively short distance from the surface. Beyond this limit no more material can be added on. Empirical formulæ have been devised to express the whole process, but need not detain us here.

As mentioned incidentally above, it must not be forgotten that the solvent is adsorbed on a surface in addition to, or even in preference to, the solute. From some salt solutions, for example, water may be adsorbed in greater proportion than the salt. This has been called "negative adsorption" from the point of view of the solute, since its concentration is lower on the surface than in the body of the solution and the latter becomes more concentrated. When pure solvent is adsorbed, as by charcoal in water, it is rather more difficult to picture the process. It is clear that the water molecules must be closer together on the surface of the charcoal than in the water phase itself. In other words, water is compressed. The problem will be met with again in connection with the imbibition of water by emulsoids.

Such compression implies development of heat. In the adsorption of gases by charcoal this may amount to fairly high values.

Another important aspect which requires further investigation is the adsorption of more than one substance from mixtures. It is to be expected that if two substances are adsorbed at the same time, there will be less of either than if the other were absent. This is undoubtedly so in many cases investigated, and is naturally obvious when the addition of a highly adsorbed substance drives off another from its place on the surface. But there is some evidence that the presence of one may occasionally facilitate the adsorption of some other. More ammonia is stated to be adsorbed by charcoal when water is also present than from the dry gas. This possibility may be of importance in the case of enzymes. Thus it is not inconceivable that a layer of adsorbed protein might be able to adsorb another protein as well as, or even better than, the bare surface of the enzyme particles.

Adsorption plays a large part in physiological phenomena. We have already obtained indications of its part in the action of enzymes, and a few other aspects may be mentioned briefly.

There are certain complex compounds which appear on investigation to consist of a mixture of simple compounds united together by surface forces. The reason why these compounds are believed not to be definite chemical individuals is that their constituents vary in relative amount according to the concentration of each particular substance in the mixture from which they are separated. The curious complex known as *jecorin*, originally obtained from the liver, but also made artificially, consists of lecithin, a protein and a carbohydrate in various relative proportions according to the mode of preparation. Many of the proteins are probably complexes of a similar kind, as are also the products of the reaction between a toxin and its anti-body. Perhaps the simplest of these "adsorption-compounds" is the precipitate produced by adding colloidal arsenious sulphide to colloidal hydrous ferric oxide, whose composition differs according to the relative proportion of the solutions mixed. This will be referred to again in a later chapter.

Adsorption plays a part in the action of some drugs which do not enter the cell but are deposited on its surface and act from that situation. It has been found that muscarine and pilocarpine are inactive after they have actually entered the cells of the heart and intestinal muscle respectively. Strophanthin itself does not enter the cells of the frog's heart at all, and it is of interest that this drug is allied to the saponins, which have great effect in reducing surface energy. There is also evidence that potassium and calcium exert their effects on muscle cells from the outside only.

It was at one time believed, chiefly owing to the

influence of Ehrlich's work, that particular constituents of cells or tissues were stained by dyes, owing to a chemical affinity between the dye and some component of the tissue. Further investigation has been unable to find definite relationship of this kind. Dyes of very varied chemical nature are found to stain a specific constituent of the cell, while the same dye can be made to stain substances of different chemical composition. At the same time, it is not yet clear what are the properties concerned. In some cases, simple adsorption appears to be the explanation. In others it is more difficult to account for the phenomena on this basis. It must be remembered that the physical properties of a surface ultimately depend on its chemical nature, and that there is a very complex interaction of forces at the interfaces between phases of various chemical composition.

The Cell-Membrane

There is another important case of adsorption which must not be omitted. I refer to the semi-permeable membrane which covers cells. It will be realized how decisive the properties of this membrane are in connection with the permeability of cells to various substances and in regulating the osmotic pressure within them. Any material within the cell which lowers surface energy will be accumulated at the surface. Certain substances, proteins, saponin, quinine and others, have been shown by Ramsden to be concentrated in surface films to a degree which exceeds their solubility, so that they are deposited in a more or less rigid form. This deposit is capable of being dissolved again if carried into the interior of the solution, even in the case of proteins to some extent, although these are apt to undergo an irreversible coagulation. According to this view of the cell-

membrane, it is a variable structure and is in equilibrium with the changing states of the cell. It is not fixed ; for example, it becomes more permeable in conditions of excitation or activity.

Suggestions have been made as to the possible means by which changes in permeability may be brought about. Perhaps the most interesting of these is that of Clowes. We have already seen that two kinds of systems of two immiscible liquids are possible ; we may have, say, an emulsion of oil in water or of water in oil. If we may compare the oil to land, we may look upon the former system as a number of islands in a large lake ; the latter as a number of small lakes surrounded by land. Fish could pass from one side to the other of the former system ; rabbits not. The opposite would be the case with the lakes surrounded by land. A layer of the oil in water system would only allow to pass through it substances soluble in water, because the water is the only continuous phase. The latter would be permeable only to substances soluble in oil. Sodium chloride would only pass through the former ; fat only through the latter. Alcohol being soluble in both oil and water, would pass through either. A cell in the resting state, a muscle fibre, for instance, is in fact impermeable to sodium chloride, but becomes so when in activity. It is always permeable to alcohol. Clowes shows also that one system can be changed into the other by the action of certain salts, such as those of calcium. This is because the difference between the surface tensions on the oil side and on the water side at the interface is of opposite direction in the case of a film of calcium soap and of sodium soap. Thus the former emulsifies water in oil, because the film is concave on the water side and encloses drops of water. The opposite is the case with sodium soap. It is not to be

supposed, however, that this hypothesis is more than a tentative one. The actual state of affairs in the cell-membrane is doubtless one of greater complexity. For example, the dimensions of the channels of continuous phase formed between the droplets of the dispersed phase, or, in other words, the size of the pores in the membrane, may be small enough to act as a mechanical sieve, similar to that of most artificial membranes.

Electrolytic Dissociation

Although the view that the electric charge on colloidal and other surfaces is due to the adsorption of ions seems to be that most favoured at the present time, there are other ways which may be of more importance in certain cases.

Suppose that we have a solution of aluminium sulphate and that we produce aluminium hydroxide in it by the addition of alkali. Aluminium hydroxide is, in the ordinary sense of the word, insoluble in water, and either becomes a colloidal suspension or falls as a precipitate. The molecules rapidly aggregate together. Consider those forming the surface of a particle. These will be dissociated and the negative OH ions tend to wander into the water, being freely soluble; but since the opposite ion is insoluble and cannot leave the surface, the OH ions are held by electrostatic attraction at a certain small distance away from the surface. The particle is thus left with a positive charge. Such particles consist of a large number of undissociated molecules covered by a layer of ions. Hardy suggests calling the whole a "colloidal ion." It is to be remembered, however, that the process of adsorption of ions may, under certain conditions, reverse the sign of the charge in such cases. Thus Powis has shown that the particles

of ferric hydroxide, if prepared in the presence of a large excess of sodium hydroxide, may have a negative charge instead of a positive one as in the usual circumstances. The great concentration of OH ions in the solution causes their adsorption to so great a degree as to neutralize and then reverse the positive charge of the surface.

Consider next a somewhat different case, that of congo-red. This throws light on that of the proteins, which are of special interest to the physiologist. Congo-red is the sodium salt of an insoluble acid which has a large complex molecule. The sodium salt forms a colloidal solution in the sense that it does not diffuse through parchment paper. Measurements of electrical conductivity show the solutions to be highly dissociated, whereas the osmotic pressure is only that of a non-dissociated compound. The only explanation of this behaviour is that the anions form large aggregates, with a minimal or zero osmotic pressure, but with the combined charges of each constituent ion. Conductivity measurements show that these "colloidal ions" have a mobility at least equal to that of simple ions. McBain's recent work on soaps has especially emphasized this point. Thus, the non-diffusibility of the salt is due to the aggregated anions. The sodium ions, of course, cannot leave the neighbourhood of their fellow ions because of electrostatic attraction. But it is probably these alone which give the osmotic pressure. The mechanism of the process will be discussed further in Chapter IV.

So far as can be made out, similar considerations apply to the proteins. But since these are amphoteric, that is, they combine either as bases, owing to their NH_2 groups, with acids, or as acids with bases owing to their carboxyl groups, the salts are of different nature according to the acidity of the solution. At a

particular value of this hydrogen-ion concentration, the "isoelectric point," dependent on the relative strength of the acidic and basic groups, the proteins are free and uncombined with either. The sodium salts are similar to congo-red, highly dissociated, the protein ions aggregated and indiffusible. The salts with acids behave in an analogous way; in this case the protein ion is the cation, with positive charge. Thus we see that the colloidal particle in the cases of congo-red and sodium proteinate possesses a negative charge owing to its composition as an aggregate of anions; in the case of the protein chloride, the colloid has a positive charge, owing to its composition as an aggregate of cations. Thus we see that there is probably no simple molecule with colloidal properties in these cases. At the isoelectric point, the osmotic pressure is very small, owing to the high degree of aggregation of the free protein.

It is true that there has been some dispute as to the source of the charge on protein particles. Some observers regard it as due to adsorption of hydrogen or other ions. But there is no doubt that true salts exist, giving rise to charged ions by electrolytic dissociation. Whether, in somewhat high concentrations of acid or base, adsorption adds further charges is not definitely known, although the effects of the ions of neutral salts suggest the probability of its being so, as will become clearer in the next chapter.

The recent work of McBain on the soaps has added further knowledge. This observer holds that in these cases, which are salts of acids of high molecular weight and thus analogous to congo-red, the anions form complex aggregates which include a proportion of undissociated soap together with water. Such an aggregate he calls an "ionic micelle." It is quite possible that the aggregates of anions of congo-red

and of proteins also contain undissociated salt and water, but we have no definite information on this point.

Electrical Adsorption

We are now in a position to consider those cases in which adsorption depends on the neutralization of electrical charges. The phenomena will be made more intelligible by the consideration of specific cases. If we take ordinary filter paper and immerse it in a dilute solution of a so-called "acidic" dye, that is one in which the coloured portion is the acid or anion, united with sodium, say congo-red, we find the paper deeply stained. But if we use analytical paper as free as possible from ash, we find that it is scarcely stained at all. On the addition of a small amount of a neutral salt, such as sodium chloride, the purest paper becomes deeply coloured. Take now a "basic" dye, in which the base or cation is the coloured part. We find the behaviour precisely the reverse. The purer the paper, the more deeply is it stained, and the depth of stain is decreased by neutral salts. What is the explanation of these facts?

In the first place, the statement of Michaelis and Rona, although accepted by Matthews in his article in *Physiological Reviews*, is most certainly incorrect. According to these observers, the staining of paper by a basic dye is due to chemical combination with inorganic salts in the paper. The facts that the presence of salts really decreases the stain in this case, and that the purer the paper the deeper the colour, are so easy to make out that I am unable to understand how the mistake was made.

We must begin with the fact that, like most chemically inert substances, paper has a negative charge in water. The origin of such charges will be discussed

presently. In a solution of congo-red we have the positively charged sodium ions and the aggregated anions with negative charges. It is clear that the former only will be attracted by the negative paper; the coloured ions will be repelled. Or we may look at the matter from the point of view of the second law of energetics. If negative ions were adsorbed, the charge on the paper would be increased, with a rise of free energy, and this does not occur unless energy be supplied from another source. But if there is present a sufficient excess of positive ions, these will first of all be adsorbed, causing a diminution or even abolition of the negative charge and removal of the obstacle to adsorption of the coloured anions. An explanation of this kind was first given by Victor Henri and L. des Bancelles in relation to the effect of salts on the staining of gelatin. Turning now to the basic dyes, where the coloured ion is the cation with a positive charge, we note that the higher the negative charge on the paper, the better will be the adsorption. Neutral salts, by reducing this charge, decrease the degree of adsorption. This is the process known as "electrical adsorption." It occurs in all cases of charged surfaces when charged particles of various kinds are present. It differs from chemical combination between ions in that it is a surface phenomenon and can be reversed by changing the sign of the charge on the surface by the adsorption of ions of opposite sign.

An interesting fact which confirms the truth of the hypothesis given, is that factors other than electrolytes but which affect the magnitude of the charge have their expected effects on electrical adsorption. For example, the charge is proportional to the dielectric constant of the liquid in contact with the surface. Water has a higher dielectric constant than alcohol, and accordingly it is found that the decrease of the

negative charge by the addition of alcohol brings about a greater adsorption of an acidic dye and a less one of a basic dye.

The degree of adsorption of various solutes by paper can be well shown by suspending strips of filter paper so that their lower ends are immersed in the solutions. The solvent rises higher in the strip than the solute does, and if this latter is coloured the difference is plain to the eye. Otherwise, it may be detected by chemical means. This method, called "capillary analysis," has been developed in detail by Goppelsroeder.

Static or Frictional Charge

The source of the charge on pure paper in pure water seems to require some further explanation, which has also its application to certain cases of colloidal particles or droplets. When two substances become charged by rubbing together, it is found that the one with the higher dielectric constant becomes positively charged, the other negatively. This may be said to be simply an experimental fact, and the full explanation of such phenomena is not forthcoming at present. Water has a dielectric constant higher than paper, or indeed than most other substances. Hence the sign of the charge on paper in water is in accordance with rule. On the other hand, it has not been shown that the adsorption of ions from the liquid medium of dispersion is insufficient to account for all the facts. It seems difficult, however, to explain the charge on drops of paraffin oil in pure water on this view, at all events at present.

Effect of Electric Charge on Surface Tension

Although the presence of an electric charge has no direct effect on the forces producing surface tension,

the net result is to decrease the effective surface tension. This being dependent on attractive forces between molecules, is opposed by the repulsion between the similarly charged elements of the surface.

The Muscle System

It is of interest at this point to examine the part played by adsorption in the phenomena of muscular contraction. It appears that the pull exerted by a muscle when it enters into activity is due to the adsorption of lactic acid or its ions at the boundary surface between some longitudinal fibrillæ and the sarcoplasm.

That surface energy is involved is shown by the facts investigated by A. V. Hill, who showed that the energy developed in a contraction is in direct proportion to the length of the fibres at the moment when they enter into activity. That it is the length, not the volume, that is concerned indicates that it is the area of some longitudinally arranged surfaces that is the factor of importance, not an osmotic process. Thus the surface tension theory of Fitzgerald appears to be the correct one. Similar facts are shown by the work of Starling to govern the adjustment of the output of the mammalian heart to increase of venous inflow, rise of arterial pressure, and the other factors which play a part. Everything may be put into a "Law of the Heart," which states that the output is in proportion to the length of the ventricular fibres at the moment of systole.

What is the nature of the complex system which possesses the potential energy liberated by a stimulus is a very difficult problem, about which we know very little. That it contains a large part of the lactic acid set free, returned by expenditure of energy derived from oxidation of a small part of it, is clear. Meyerhof's

recent work indicates that this part of the system is glycogen, synthesized from lactic acid.

Further details are beyond the scope of this book, and, indeed, more work is needed before the whole mechanism can become intelligible.

CHAPTER III

PRECIPITATION AND PEPTONIZATION

WE have seen that colloidal particles, except under special conditions, possess electrical charges and are to a large extent prevented from aggregating together and falling as precipitates by the mutual repulsion of these charges.

It was known to Faraday that the addition of a neutral salt, such as sodium chloride, to colloidal gold would cause its precipitation. The phenomenon was further investigated by Linder and Picton, by Hardy and by Schultze. It was found to be due to the neutralization of the charge by the adsorption of the oppositely charged ion of the salt added, which ion was carried down by the colloid. The fact that it was always the ion of opposite sign to the colloid that was responsible became known as "Hardy's rule." From the point of view of the theory of probability, it will be clear that the encounter of a colloidal particle with a bivalent ion would impart double the charge that one with a univalent ion would. Hence bivalent ions are as a rule more effective than univalent, and trivalent ions than bivalent.

But there are two circumstances that show that the effect is not quite so simple as just described. In the first place, the maximum precipitation is not exactly coincident with the point of absence of charge, the "iso-electric point," as Hardy called it. The particles

in many cases still possess a slight charge at the salt concentration of optimum precipitating effect. It seems evident that the repulsion due to this slight charge does not prevent the action of other forces tending to cause aggregation and deposition. But the explanation is not yet clear. In the second place, if the electrical attraction of opposite charges were the only force to be considered, all univalent ions should be equally active and always inferior to bi- or trivalent ions. Now, while different colloids do not all show the phenomenon in the same degree, there is abundant evidence that the adsorption of ions is determined by forces similar to those responsible for ordinary adsorption. We cannot say as yet why a particular colloid, such as that of sulphur, is one hundred times more sensitive to cæsium than to lithium, or why it is about equally sensitive to the univalent cæsium and the bivalent magnesium, or, in other words, why it adsorbs cæsium so much more than it does lithium or magnesium. Ferric hydroxide shows this property to a much less degree than sulphur does; but it is clear that our knowledge of the forces producing adsorption is not yet complete. If, however, they were simply chemical, as is held by Loeb to be the case with proteins, it is difficult to see why lithium and cæsium should differ to so large a degree. The phenomena certainly suggest that in the case of proteins, which are also aggregated molecules, other forces than purely chemical ones must be considered. To this point we shall have occasion to return in Chapter IX.

The colloids known as emulsoids, amongst which are the above-mentioned proteins, are much less sensitive to the precipitating action of salts than the suspensoids are. But careful investigation shows that this is merely a matter of degree. The effect of the neutralization of electric charge is, in the case of the

amphoteric proteins, liable to be confused with changes of solubility due to formation of chemical compounds with the ions of the salts added. But there is no doubt that the neutralization of charges plays a part independently of combination in molecular proportions, as especially shown by the effect of trivalent lanthanum salts, which are effective in small concentration, and also by the precipitation of emulsoids by oppositely charged colloids, such as arsenious sulphide or ferric hydroxide respectively. The latter is much used for the removal of protein from serum. This phenomenon is one aspect of the mutual precipitation of oppositely charged colloids, to be referred to presently.

In general, the lower sensibility of emulsoids to the action of electrolytes seems to be related to the smaller contrast of composition between the two phases than is the case with the solid suspensoids free from water.

The well-known "salting out" by the addition of relatively large amounts of neutral salts, even those of univalent ions, is not directly an electrical effect, but appears to be due to removal of water from the internal phase. Hatschek has pointed out the importance of this effect of electrolytes in altering the distribution of water between the two phases of an emulsoid system. Actions of this kind are of much importance in intracellular processes.

That we are not dealing merely with a chemical interaction in the phenomena under discussion, nor on the other hand with an electrical neutralization only, is shown by the different effects of ions of the same sign of charge. In the swelling of gelatin, for example, sodium chloride retards, while sodium acetate accelerates. The thiocyanate ion has an especially marked influence in increasing the taking up of water. The question will come up again in discussing the phenomena of imbibition. It is evident that specific adsorp-

tion plays a large part, as in the precipitation of suspensoids, but the explanation of the process as a whole is not yet clear.

Protection

Faraday discovered that the addition of a trace of "jelly" to his gold solutions made them much less sensitive to precipitation by salt. This result is frequently attributed to adsorption of the emulsoid by the gold particles, thus conferring on their surfaces the less sensitive properties of the emulsoid. Various proteins differ in their powers of protection, and their relative capacities under the same conditions are called their "gold numbers." But, as Freundlich has pointed out, the most insensitive colloids are not necessarily the most effective protectives, so that again it appears that adsorption in different degrees plays its part.

The fact has its practical importance in the preparation commercially of inorganic colloids in a stable form, very little sensitive to electrolytes. The presence of these protectives, which frequently have themselves a marked physiological action, renders reports of therapeutic action, supposed to be due to the inorganic colloids, of no value, unless controlled, and information given of the effects of the protective present. Peptone and similar compounds, for example, are sometimes used. Silicic acid in the colloidal form is also an efficient protective and has been used commercially. According to the work of Gye, silica has toxic properties, so that it should not be used in therapeutic colloids. Reports of cases in which commercial preparations made by a secret method were used have no value as evidence. The absence of adequate comparison with other methods of treatment is frequently to be noted. It should be pointed out that the addition

of protectives to such colloidal solutions is for the purpose of maintaining them in a satisfactory dispersed state and to avoid the precipitation brought about by foreign electrolytes. If sodium chloride is added to make the solutions isotonic with blood, protection is obviously necessary. But the amount of the colloidal solution injected is usually so small and should be given at so slow a rate that this isotonicity is unnecessary. Indeed, by very careful preparation, the majority of metallic colloids themselves may remain stable for a sufficiently long time. The suggestion that a protective is needed to avoid precipitation by the salts of the blood is incorrect, because the proteins of the serum are sufficient protection when the colloidal solution has entered the circulation.

Mutual Precipitation

Since an electrically charged colloid can be precipitated by an ion of the opposite sign, it is natural to expect that an oppositely charged colloid would have the same effect. If the appropriate relative amounts are chosen, it is found that both colloids are precipitated together as an adsorption compound, devoid of charge. It is sometimes stated that the precipitate is soluble in excess of either colloid. This is correct in so far as the precipitation is incomplete, but the actual state of affairs, as can readily be seen by using colloids of different colour, such as the negative arsenious sulphide and the positive ferric hydroxide, is that an adsorption compound of different relative proportion of the two is always formed. If the preponderance of one of the two is great enough, a sufficient charge is left to maintain the stability of the system, the compound particles remaining in suspension.

This is perhaps the most suitable place to refer

briefly to the production in the living organisms of what are called "precipitins" for foreign proteins. When one of these, say egg white, is introduced into the body of a rabbit, there is developed in the blood a capacity of precipitating a solution of egg white when this is mixed with the serum. This is one of the varieties of "anti-body" formed to neutralize foreign toxic substances. Whether precipitins are actual chemical individuals is not definitely known, and the same statement applies to the other anti-bodies in general, be they "agglutinins," "hæmolysins," or others. All that we can say is that the serum of the immunized animal has acquired properties of neutralizing in some way the effect of the "antigen" whose introduction gave rise to the development of these properties. It is necessary to point out that there is no evidence that "anti-enzymes" are formed in this way. The solutions of enzymes used as antigens contain proteins as impurities, and precipitins may be formed for these. Enzymes themselves are not of protein nature, although they are colloids, and it seems that it is only to proteins that anti-bodies are formed. There are, however, certain substances present in serum, egg white, etc., which retard the action of enzymes, but these are not produced in response to the injection of enzymes. Their action is probably to be explained either by their aggregating effect, or more probably by their adsorption on the surface of the particles of the enzyme and the displacement of the proper substrate thereby (see p. 25). The way in which precipitins themselves act is not clear.

Peptization and Emulsification

"Peptization" is the term used by Graham to describe the process by which a lump of solid material

is converted into a colloidal solution—is dispersed in a liquid medium, as we might say at the present time.

Before proceeding further, it may be well to direct our attention to the way in which surface tension is concerned in the stability of a colloidal solution. If any two particles or droplets in the course of their Brownian movements actually hit together so as to fuse, the total surface is decreased and consequently the free energy of the system. The second law of energetics leads us to expect that this will happen, and the object of an electrical charge or other stabilizing agent is to prevent the actual encounter, or, by means of a film on the surface, prevent coalescence when the particles do come into contact. Thus, sometimes the addition of a substance which lowers the surface tension on the particles adds to the stability of a colloidal solution. The surface tension being low, is not, as it were, so eager to become lower still. But in the more usual cases, and especially in the preparation of emulsions of liquids such as oil or paraffin, the formation of a soap film on the droplets is the effective agent in preventing coalescence.

Many substances can be dispersed by giving to the particles electric charges, as in the peptization of metallic oxides by potassium hydroxide. In some of these cases, as in that of alumina, the result is for the most part a solution of potassium aluminate. But in others, as in chromium hydroxide, the whole of the chromium can be filtered off by pressure through a collodion membrane, so that it is dispersed as particles not in combination with the alkali. What are the conditions that decide which shall take place is unknown.

Certain dry solids, particularly the proteins, become dispersed merely by adding water. Presumably, the necessary materials to form the ions which confer the

charge are present from the beginning in the original solid.

In the coagulation of some proteins by heat, such as egg white, the particles under certain conditions are aggregated together in such a way that they can readily be redispersed by an electrolyte conferring an electric charge. Under other conditions, they cannot be redispersed, or only with difficulty. Chick and Martin have suggested that the difference is as represented in Figure 9. The state in A can easily be redispersed, the second B only with difficulty. In B the particles have so far fused together as to form a



FIG. 9.—Different Forms of Aggregation in Coagulated Egg-White.—Chick and Martin.

continuous large particle. A similar explanation probably holds in the case of “salting out” by high concentrations of salts. If the precipitate be quickly suspended in water, it becomes redispersed as at first; if allowed to remain in contact with the strong salt solution, this cannot be done. The protein becomes “insoluble,” as usually expressed.

The Clotting of Blood

Although to discuss this problem in any detail would require much more space than is appropriate here, it is obvious that the process is essentially a

change in the physical state of certain colloids present in the plasma. An unnecessary degree of complexity has been introduced into the numerous theories of clotting by the assumption of a large variety of participants, each supposed to be a definite chemical compound and given a name. There is evidence, indeed, that the investigation of the phenomena with due regard to the colloidal factors involved will simplify the theories. The recent work of Pickering, which shows that the views put forward by Wooldridge are in the main correct, may be especially referred to. The fact that clotting is brought about by any foreign surface wetted by the plasma, indicates that surface forces play a large part.

Structure of Protoplasm

While it is obvious by ordinary microscopic observation that the contents of a cell form a highly heterogeneous system, it needs the application of intense dark-ground illumination to show anything but a clear liquid in the pseudopodia of *amœba* or a leucocyte. Under such conditions, however, the protoplasm can be seen to be filled with a multitude of shining points in Brownian movement. It should be kept in mind in all cases where colloidal solutions are examined in this way that diffraction discs of light are much larger than the actual particles themselves, so that these appear to be much more crowded than they really are. Even the simplest protoplasm is thus a colloidal system, and the existence of Brownian movements shows that it has the properties of a liquid. The micro-dissection experiments of Chambers also indicate its liquid nature. A needle drawn through it, for example, does not leave a track behind and experiences no notable resistance. Under the influence of electrical stimulation, and also

in such states of activity as mitosis and after fertilization, there is a change in the direction of increased viscosity and cessation of Brownian movements, indicating conversion to a more or less jelly-like state. It is interesting to note that this change, which seems to be an indication of activity, is not present in the pseudopodium, so that the protrusion of this process is not associated with an excitation of the organism, and is probably brought about by local lowering of surface tension, due to the influence of something in the environment.

A very significant fact that is not always sufficiently realized by histologists, is that the methods of fixation used cause precipitation of colloids and the production thereby of various forms of aggregates—granules, fibrils, etc. The materials from which these structures are made were present in the living state as the fine particles of colloidal solutions. The different forms of network, etc., produced by the action of fixing reagents were investigated by Hardy, whose work is apt to be overlooked. There seems to be no doubt that the reticular structure which has been described as present in living protoplasm is due to inadequate methods of illumination and consequent confusion by diffraction images. As an illustration of the importance of examining the living cell, we may take the work done by Mott and by Marinesco, independently, on the cell-bodies of the spinal neurones. Under dark-ground illumination, these are seen to be filled with a colloidal solution, containing particles in vigorous Brownian movement. Nothing was seen of either Nissl granules or neuro-fibrils, which must be produced by coagulation and aggregation of the cell colloids when fixing agents act on the protoplasm.

CHAPTER IV

OSMOTIC PRESSURE

THE conception of osmotic pressure is a difficult one to grasp, and indeed the precise cause of it is still a matter of dispute. Perhaps we may best approach it by considering what happens when we place a solution of sugar inside a bag made of a material whose pores are large enough to allow water molecules to pass through, but too small for the larger ones of sugar. Let the bag be immersed in water, after tying into it a tall glass tube open at the upper end. We then find that water goes into the bag, raising the level of the solution in the glass tube. Thus, the solution becomes diluted, and at the same time pressure corresponding to the height of the column in the tube has been developed. The mechanism of the process may be looked at, from the point of view of certain considerations of Van der Waals, somewhat as follows. The whole surface of the membrane of the bag on the outer side is covered by water molecules; on the inner side, part of the area is occupied by molecules of sugar, so that the proportion occupied by water is less than on the outside. The molecules of water, by their kinetic movements, hit against the membrane from both sides and, since this membrane is permeable to water, those molecules that hit pass through. If water alone were present on both sides, the number passing in one direction would be equal to that passing

in the other direction, because as many on the average hit one side as the other. But when there is some solute present in the water, inside the state of affairs is different. While the molecules of water coming from the outside find the whole area passable to them, those from the inside find only a part of it passable, because part of it is occupied by molecules of sugar. Hence, more water molecules pass inwards than pass outwards in a given time. Water accumulates inside, by "osmosis," until the pressure is great enough to force outwards by filtration sufficient additional water molecules to balance the excess of those entering. There are other ways of explaining the process, but that given is perhaps the most intelligible. It will be seen that the osmotic pressure developed is proportional to the number of molecules, or corresponding units, ions or aggregates, in a given volume—that is, to the concentration of the solution.

In its physiological bearing, osmotic pressure may be of importance either on account of the pressure itself, as in the case of plant cells, or on account of the water attracted by it, as in the process of secretion, or in certain phenomena connected with the blood, to which attention will presently be directed.

It will be clear that the possibility of osmotic phenomena depends on the presence of an appropriate membrane, which must be permeable to water, not to the dissolved molecules. It will further be evident that the pores of the membrane may be of such a size as to allow small molecules like those of sodium chloride to pass through, but not large enough for the aggregated molecules of colloids. The membrane covering individual cells and blood corpuscles is impermeable to salts, glucose, etc.; that forming the walls of the capillary blood vessels is permeable to these, but impermeable to colloids. Hence, the osmotic pressure

of inorganic salts and sugar manifests itself in the former case ; only that of the colloids in the latter.

Now it is found that while a solution containing single small molecules is capable of exerting a high osmotic pressure, only a certain kind of colloidal solution has any perceptible one at all. The suspensoids, for example, have only an infinitesimal osmotic pressure, although on the basis of the number of molecules present, they should have a high one. Why is this ? Simply because these molecules are joined up together in large groups, each of which acts, osmotically, only as one molecule. Such groups, as we have seen, may contain as many as one thousand molecules, so that the osmotic pressure developed would be only one-thousandth part of that produced if they were separate from one another in true solution.

But, on the other hand, we find that there are some colloidal solutions which manifest a fairly high osmotic pressure. These include many of the dyes, the proteins, the soaps in certain ranges of concentration, gum arabic, etc. When we examine the nature of these, we find that they are mostly salts of an inorganic base or acid, such as sodium or chlorine as cation or anion respectively, with a large organic ion as the anion or cation. Thus, the soaps are sodium or potassium salts of oleic acid ; the dye, " night blue," is the hydrochloride of a large organic colour-base. Proteins may be either salts with sodium or with chlorine, according to the hydrogen-ion concentration of the solution. Measurements of electrical conductivity show, further, that these salts are highly dissociated, so that there is a fairly large concentration of the inorganic ion present in the solutions. Since the osmotic pressure is shown even when the membrane is permeable to these ions, the question arises as to whether they do or do not play their part in the osmotic

pressure. The reason why, although able to pass through the membrane, they do not escape, is clear. The electrostatic attraction of the opposite ions is so great that the diffusible ions can only leave their neighbourhood for a very short distance. This distance, however, is sufficient to enable the diffusible ions to form a layer on the far side of the membrane, thus giving it a charge of the corresponding sign; in the case of congo-red, the sodium ions give a positive charge. It may be remarked, incidentally, that a similar process seems to be the cause of the electrical phenomena of living cells in general.

But do these diffusible ions behave, osmotically, as if the membrane were impermeable to them? The answer to this question is by no means obvious at first sight. Evidence of various kinds, however, both theoretical and experimental, shows that they do. Measurements with congo-red which do not involve a membrane permeable to one ion only, as, for instance, vapour pressure measurements, in which the air may be looked upon as a membrane impermeable to both ions, give the same values as those obtained with parchment paper, which is permeable to the sodium ion. Perhaps the simplest way to realize the state of affairs in the latter case is to imagine that these diffusible ions can only be held back by the indiffusible ions if these latter are able, so to speak, to put their feet on the membrane to give them a support to pull against. Thus, the membrane must be subject to the pressure required to oppose the osmotic pressure of the diffusible ions, and this will show itself as a pressure in the liquid within the membrane. But, further than this, it appears that the osmotic pressure of those colloidal solutions which possess one is, to all intents and purposes, due solely to the inorganic ions. Measurements which I made of the electrical conductivity

compared with the osmotic pressure of congo-red solutions showed that the osmotic pressure was not nearly high enough to permit the view that the large organic ions played any important part. To explain this fact, I adopted the hypothesis that these anions are largely aggregated, so that their osmotic pressure would be relatively small. But it was necessary to suppose, to account for the electrical conductivity, that they retained the sum of the charges of their component ions. They must also possess a mobility practically equal to that of simple ions. A view similar to this was subsequently proposed for proteins, and in a slightly modified form as "ionic micelle," by McBain for soaps. The ionic micelle, however, contains undissociated salt in addition to water. It is quite possible that the aggregated anions of congo-red may also contain undissociated salt and water. In the case of soaps, McBain states that the observed osmotic pressure is all to be accounted for by the sodium ions.

The case of proteins is of sufficient physiological importance to warrant a special discussion in Chapter IX.

We may venture, I think, to make the general statements that the osmotic pressure of colloidal solutions, where present, is almost entirely due to the inorganic ion of an electrolytically dissociated salt, and that the opposite ion is aggregated to such an extent as to play a negligible part in the production of the pressure.

This being the case, it is clear that, even when the colloidal properties of a substance appear to be due to its large molecule, there are always present aggregates which have surface properties, and that any theory based on the simple chemical behaviour of molecules can only account for the facts observed under

particular conditions. In other words, the probability of adsorption, amongst other things, must be kept in mind.

On the other hand, the osmotic pressure shown by colloidal solutions cannot be explained as due entirely to adsorbed electrolytes or ions. An adsorbed ion forms, for the time being, an integral part of the colloidal aggregate on whose surface it is held. It cannot, therefore, be osmotically active to any greater extent than the aggregate to which it has become attached. In practice, it is found that the osmotic pressure of the colloidal solutions in question becomes progressively higher as other foreign electrolytes are removed by dialysis. While present, they reduce the effective osmotic pressure by the Donnan equilibrium or membrane effect to be described below.

The reader may remember that in an earlier page of this book reference was made to the evidence of a slight degree of true solubility in the case of proteins—that is, presence of the protein itself in single molecules. Should this be so, it is clear that a certain very small “genuine” osmotic pressure might be present in such solutions. But it would be too small to detect, since the solubilities in question do not appear to exceed a few milligrams per litre.

Donnan Equilibrium

An important question concerns the effect of electrolytes on the apparent osmotic pressure of colloids which are themselves salts. It was found by Donnan and by myself independently, that if sodium chloride was added to a solution of congo-red in an osmometer, although the membrane was freely permeable to the sodium chloride, this did not distribute itself equally on the two sides of the membrane. The concentration

was always higher in the solution free from congo-red, the difference being the greater the higher the concentration of the dye in proportion to that of the sodium chloride. Donnan deduced the result from thermodynamic considerations. We may, perhaps, look upon it as a result of the effect of the dye salt on the dissociation of the sodium chloride. Since there are no forces preventing equal concentration of undissociated sodium chloride on the two sides of the membrane, this fraction of the total salt present will be the same in both places. But since the salt is less dissociated in the presence of the congo-red, the fraction of undissociated salt will be greater on the inside and the total amount less. The numerical proportions are given in the formula worked out by Donnan, which will be found on pages 309 to 322 of the second volume of Lewis' *Physical Chemistry*.

It will be realized that the greater concentration of the sodium chloride on the one side gives to this side a higher osmotic pressure than the other, a fact which will make the actual value of that of the colloid as measured appear less than the real value. In the case of congo-red, it seems that the difference of concentrations of sodium chloride on the two sides of the membrane is enough to account for the depressant effect; but, according to Sorensen, in the case of egg albumin, one must assume an increased aggregation of the colloid as well. Such aggregation may be regarded as an incipient precipitation in the sense of the phenomena dealt with in the preceding chapter.

Ultra-filtration

In order to separate diffusible substances from colloidal solutions, the process of dialysis, as described on a previous page, is often used. But this is a lengthy

process and, unless done under a pressure on the solution equal to its osmotic pressure, dilution occurs. If the pressure thus applied is greater than the osmotic pressure, water is pressed out, along with the substances held by it in true solution, or, more correctly, with those solutes which are small enough in their dimensions to pass through the pores of the filter. By increasing the pressure to values as high as the membrane will bear without tearing, the filtration may be made fairly rapid. An analysis of the filtrate gives the composition of the diffusible phase of the colloidal solution, apart from that of the colloid phase and its adsorbed ions or compounds. The process is known as ultra-filtration, and was first used by C. J. Martin, who supported a membrane of gelatin by impregnating a porous clay filter with a solution of it. Collodion membranes can be made of different degrees of permeability by appropriate treatment, and are now generally used, sometimes supported on a perforated plate.

Physiological Bearings

There are two physiological phenomena in which the osmotic pressure of colloids plays an important part.

The first of these concerns the formation of urine. The evidence is now overwhelming that the first stage in its production is a filtration in the glomeruli of the kidney, by which the colloidal constituents of the blood are kept back, while the crystalloidal or diffusible constituents pass into the filtrate—an ultra-filtration, in fact. Thus the membrane of the blood vessels here as elsewhere is permeable to crystalloids, impermeable to colloids. Suppose that the colloids of the blood were such as to have no osmotic pressure. There would then be no force attracting water or opposing

its separation by arterial pressure. But, as was shown by Starling, these colloids have an osmotic pressure of about 35 to 40 mm. of mercury—not a great value, but significant when compared with the available filtration pressure as present in the arterial system. This osmotic pressure represents the force with which they attract water, and if it is not opposed by a greater pressure forcing water out, formation of urine would cease, as is found to be the case when the arterial pressure becomes low. If the osmotic pressure of the colloids, which are practically all proteins, is reduced by diluting the blood, a lower arterial pressure suffices for filtration; or if the arterial pressure remains the same, the excess of pressure available for filtration is greater, and more rapid production of urine occurs (diuresis).

In the capillaries of other parts of the body a similar filtration takes place; but as the current of blood passes along these vessels to the veins, the filtration pressure sooner or later falls to a value less than the osmotic pressure of the colloids of the blood, so that these proceed to absorb the fluid exuded on the more arterial side. The part left unabsorbed is known as *lymph*. Here again dilution of the blood by a solution containing only crystalloidal substances causes more rapid filtration than can be balanced by reabsorption, œdema of the tissues results, and the fluid added, say by an intravenous injection of saline, is quickly lost from the circulation. For this reason, the writer introduced a fluid, known later as “gum-saline,” in which gum acacia, a colloidal substance, was added in sufficient amount, about 6 to 7 per cent., to give a colloidal osmotic pressure equal to that of the blood colloids. Such a solution leaves the osmotic pressure of the blood unchanged, the resistance to filtration is normal and the fluid is retained in the circulation,

provided that the properties of the capillary wall remain normal.

Turning to true *secretion*, a complex precursor is found to have been stored in the cells by expenditure of energy derived from the oxidation of glucose. This precursor is probably of colloidal nature. When the gland is stimulated to action, this substance is split up into smaller molecules, which, owing to their increased osmotic concentration, attract water into the cell. But, owing to the becoming permeable of that part of the cell membrane which is in relation with the duct, the water, carrying with it certain solutes, such as the specific contents of the secretion, passes into the duct, while more is drawn in from the lymph and blood capillaries at the opposite end of the cell. The process can be imitated by taking a glass tube, closed at one end with parchment paper and at the other end with filter paper, filled with congo-red solution and immersed in water. The parchment paper is impermeable to the dye, while the filter paper is permeable, and it will be seen that a current of water washes the dye out through the filter paper.

CHAPTER V

VISCOSITY

IN any liquid there is friction when any one molecule rubs against another. This occurs when a liquid flows through a tube, because the layer in immediate contact with the wall is held at rest, while there is a more or less narrow layer in which the velocity increases from zero to a maximum. The parts of this layer therefore move over one another and produce friction. The existence of this friction introduces a resistance to the ease of flow, so that under a given pressure the amount of liquid passing through the tube in a given time is inversely proportional to the internal friction of the liquid, or, as it is often called, its "*viscosity*."

When the liquid contains substances dissolved in it, or particles in suspension, the viscosity is increased. One may represent the result as being due to that part of the lumen of the tube occupied by the liquid as being reduced by the presence of the other material. Thus, the effect is equivalent to the use of a narrower tube, in which the fraction occupied by the layer subject to internal friction takes up a larger proportion of the whole than in a wider tube. Figure 10 will assist in making this clear.

In the living organism, the problem of viscosity meets us chiefly in the case of the blood. It is owing to this property that resistance is experienced in the peripheral arterioles, and the heart enabled to maintain

a steady and fairly high blood pressure in the larger arteries. Dilution with a liquid of lower viscosity than itself naturally diminishes that of the mixture. Rise of temperature also decreases viscosity. In the case of emulsoids and of blood, the temperature coefficient is a high one.

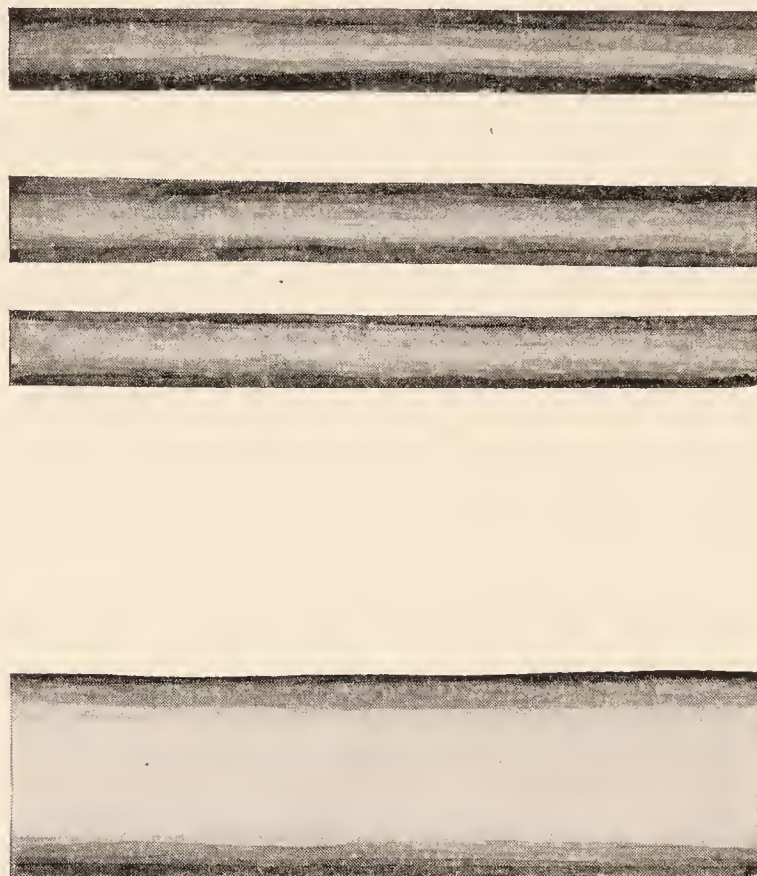


FIG. 10.—The Relative Proportion of the Lumen in Narrow and Wide Tubes taken up by Liquid exposed to Internal Friction.—Bayliss, *Introduction to General Physiology*. Longmans.

Incidentally, it may be remarked that the temperature coefficient of a process cannot be used to decide whether it is chemical or physical. That of some physical processes is practically identical with that of certain other chemical reactions.

By far the greater part of the viscosity of blood is due to the corpuscles. That of the serum is about twice that of water, whereas that of whole blood is six or seven times that of water. This being so, a point

of interest arises in connection with the laking of blood. Careful experiments show that when this is brought about without the introduction of foreign substances, say by freezing and thawing, little or no change in viscosity occurs. It is only when a hæmolytic agent such as saponin is used that the viscosity rises, and even here, as the work of my son, L. E. Bayliss, has shown, the corpuscles are almost all hæmolysed by concentrations of saponin which are too low to cause an appreciable rise in viscosity, which appears to be a secondary dissolving or swelling effect of the higher concentrations on the stromata, which have given up their hæmoglobin without becoming dissolved.

It will be realized that when hæmolysis is brought about simply by causing the corpuscles to give up their hæmoglobin, no change in the total concentration of solid matter occurs. It is therefore interesting to note that the result is in agreement with certain formulæ relating viscosity with concentration in the case of colloidal solutions. We may turn to these expressions for a moment.

Comparatively simple formulæ have been found to satisfy the experimental results when the tube through which the liquid flows is sufficiently wide not to cause deformation of the suspended particles, and when these are spherical and not in too high a concentration. Thus Einstein gives the following expression :

$$\eta = \eta_0(1 + k\phi)$$

where η is the viscosity of the colloidal solution, η_0 —that of the pure medium of suspension (water, etc.), ϕ —the total volume of colloidal matter in unit volume and k —a constant varying from 1·5 to 4·75, according to the particular case. It had been suggested that the larger value of the constant required in some cases is due to deviation from sphericity of the particles, but

the mathematical investigation of Jeffery shows that this would have the opposite effect.

Arrhenius prefers a logarithmic formula :

$$\log x = \theta c$$

where x is the viscosity of the solution, relative to that of the liquid medium of suspension, c —the percentage of volume occupied by the suspended particles and θ —a constant.

If there were available a simple and accurate method of determining the viscosity of blood, it would be a rapid and convenient means of detecting changes in the number of red corpuscles in various pathological states. Bazett finds that if θ in the formula of Arrhenius be given the value 0.00773 and the results be multiplied by the viscosity of the plasma, the viscosity of various dilutions of corpuscles with plasma is correctly given.

It will be noticed that in both the formulæ given the viscosity depends on the total amount of suspended material present, so that if aggregation occurs without change in the distribution of water between the two phases, the viscosity will be unaffected. Conversely, if a rise in viscosity occurs, it means that water has entered into the solid particles, and if it falls, that water has passed out of the solid phase into the external phase. In such cases there is an actual change in the concentration, because the water associated with the particles or corpuscles forms part of the dispersed phase, not of the continuous or liquid phase. The same considerations apply when the water is adsorbed or in any way immobilized.

When colloidal solutions are so concentrated that deformation of the suspended particles takes place in the flow through a tube, another formula, due to Hatschek, is required. A suspension of solid particles,

if not too concentrated, has a viscosity not much greater than that of water, and the viscosity is proportional to the amount of solid matter present. When we have to deal with emulsoids, however, in which the internal phase is semi-liquid and deformable, so that

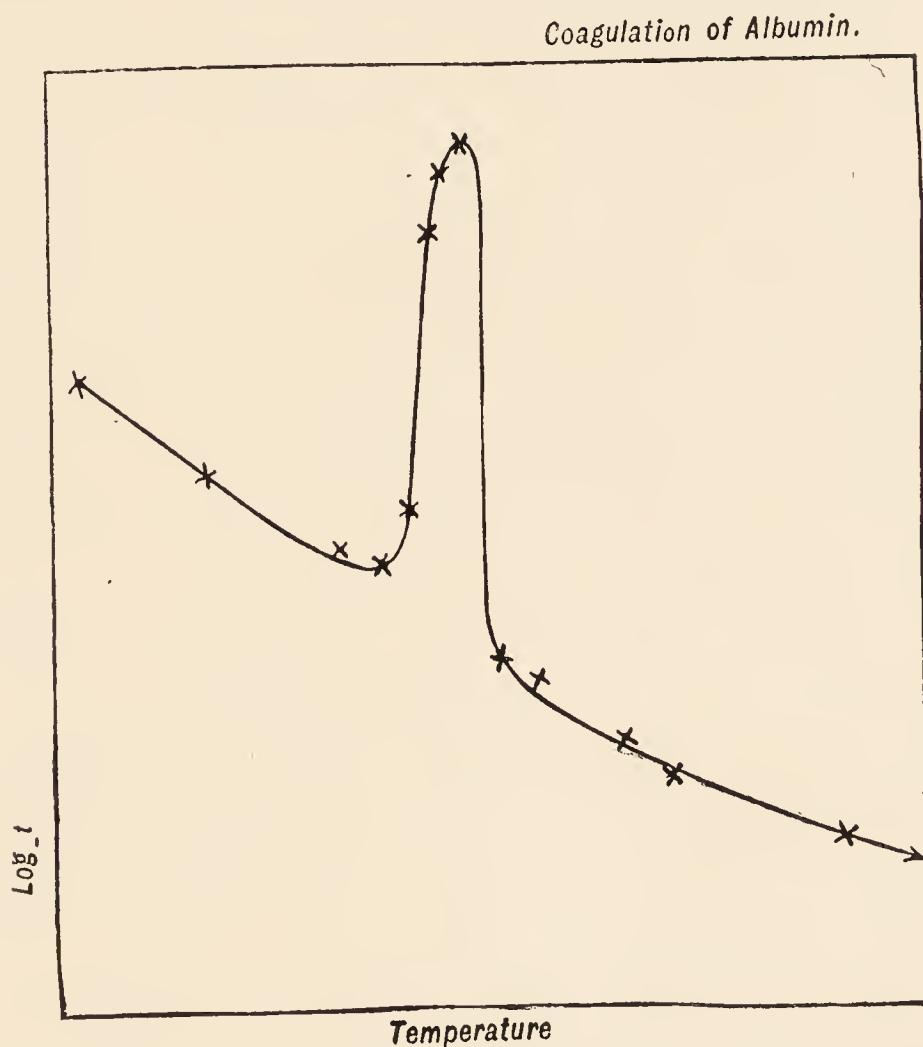


FIG. 11.

there is shear when the system is caused to flow, it is found by Hatschek that the following formula applies :

$$\eta = \frac{\sqrt[3]{A}}{\sqrt[3]{A} - 1}$$

where $A = \frac{\text{volume of whole}}{\text{volume of dispersed phase}}$. As Hatschek points out, shearing of liquids in which the droplets of the dispersed phase are so numerous as to be in contact and to form polyhedra, implies a deformation with

increase of surface and its energy. The total surface is enlarged, and the energy required to do this appears disguised as viscosity.

A rather remarkable change in viscosity was found by Wo. Ostwald to occur in the process of heat coagulation of such proteins as egg-white. When this is heated, a regular and progressive decrease of viscosity occurs from 50° to 57° . At $57^{\circ}5$, just before the appearance of turbidity, a large increase takes place, which, at 60° , gives place to an equally steep decrease. Above that temperature, the curve follows a continuation of its original course, as if nothing had happened in the meantime (Fig. 11). It seems evident that something in the nature of a structural formation occurs in the process of coagulation, by which the system as a whole approximates to a solid. Or it may be that a large volume of water is taken up temporarily into the dispersed phase.

With certain emulsoid systems a very rapid increase in viscosity occurs with concentration. In that of agar, the viscosity increases from that of water to several thousand times this value as the concentration rises from 0 to 1 per cent. This is a common phenomenon with emulsoids above a certain concentration, varying with the chemical nature of the internal phase. The particular concentration at which this rapid rise of viscosity occurs may be taken as a measure of the affinity for the dispersion medium. Above this point, the latter is taken into the colloid in relatively large amounts, so that the effective concentration rises at a relatively great speed.

CHAPTER VI

THE SURFACE TENSION OF COLLOIDAL SOLUTIONS

THE general question of the surface tension at the interface of contact between liquids and gases, solids or other liquids, has been considered in previous pages, together with its bearing on the stability and precipitation of colloidal solutions.

There is not much to be said with respect to the surface tension of colloidal solutions themselves, as manifested at the surface in contact with air or other phase. Like the majority of substances dissolved in water, those in the colloidal state also lower the surface tension to a greater or less extent.

The most important consequence of this property, from the physiological point of view, is that they will be concentrated (adsorbed) at interfaces of contact. Thus the proteins tend to form films at the free surfaces of their solutions. This fact, in all probability, plays a part in the production of the membrane on the surface of cells, a structure which has so much influence on the behaviour of these cells.

The action of gelatin and other proteins in protecting suspensoid colloids from precipitation by electrolytes is probably due to the deposition of a layer of emulsoid on the surface of the particles, as described above.

The lowering of surface tension by certain colloids, such as soap, explains their detergent action. The

dirt, usually more or less oily, becomes peptized by the deposition of soap films on its particles, passes into colloidal suspension and is washed away.

A further fact of significance in several respects was first investigated by Ramsden. Many colloids and certain substances in true solution are concentrated on surfaces to so large a degree that they form films of a more or less rigid nature, approximating to solids. They can thus be removed almost entirely from solution by shaking with air, being deposited on the surfaces of the froth.

It is necessary in the usual methods of measuring surface tension to take due account of this concentration in the surface. These methods are static—that is, they determine the surface tension of a solution after the surface has remained at rest for a time. They differ from the dynamic methods, such as that of the length of waves formed on the surface and continually renewed, in that they allow the surface concentration time to become complete, whereas the dynamic methods measure the surface tension of the solution apart from this factor, that is, in its actual concentration. Consider the “stalagmometer” method, where the size of drops issuing from a small orifice is determined by the number of drops given by a known volume of the solution, compared with that given by water. It is necessary for accuracy to allow these drops to form very slowly, so that the surface condensation shall have time to be complete before the drop falls. Although adsorption itself is very rapid when the material has actually arrived at the surface, it is clear that time is needed for sufficient material to diffuse to the surface from the body of the drop. The method by which the force necessary to draw a wire loop out of the surface is measured, as in that of Searle, enables this source of error to be eliminated with ease. Lecomte du Nouy

has recently devised an improvement of this method by which the force is measured by applying a known torsion to the wire (Fig. 12). A large number of observations on serum can be made in a short time by this apparatus.

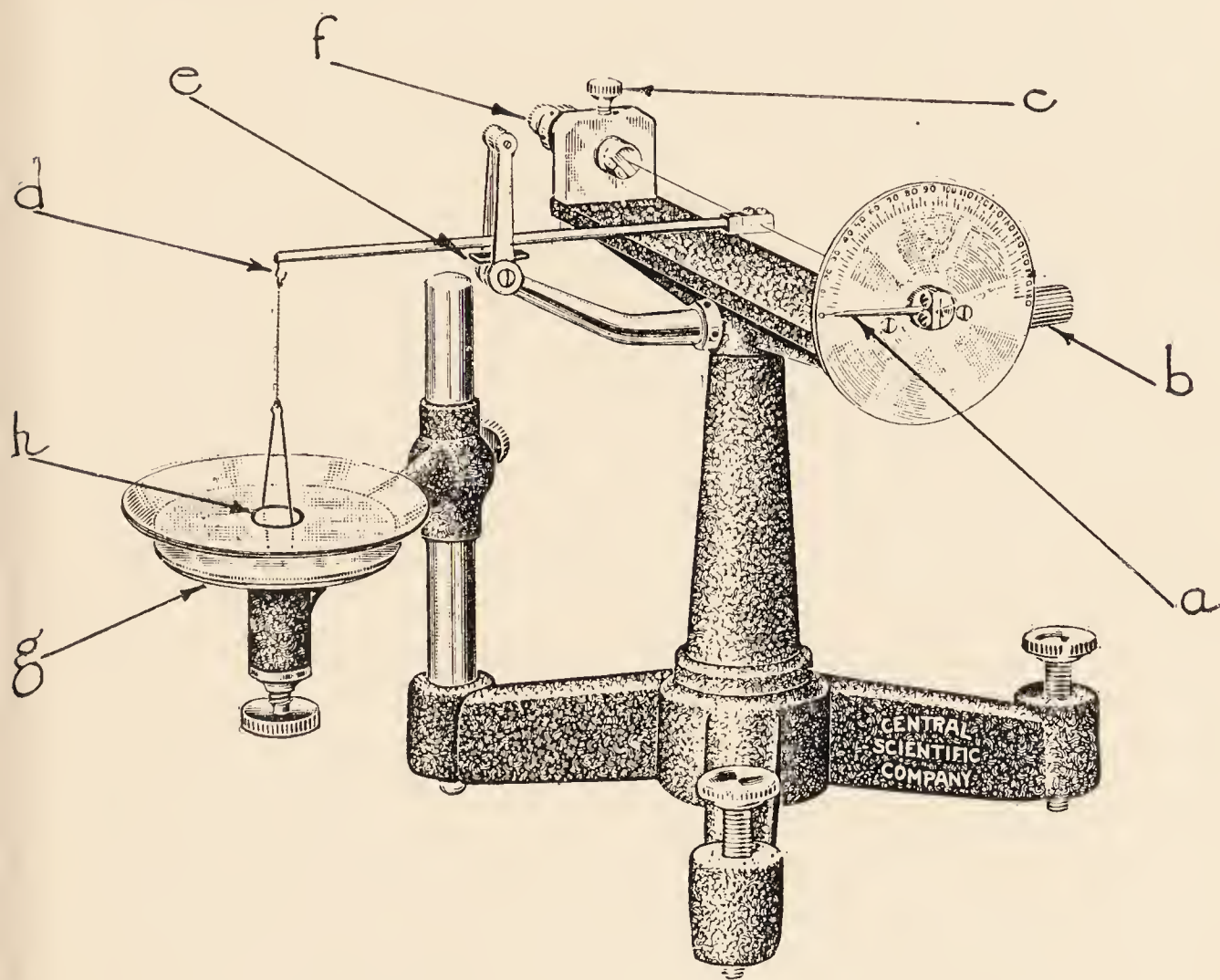


FIG. 12.—Lecomte du Nouy's Apparatus for measuring Surface Tension.

(As supplied by Central Scientific Co., Chicago.)

Lecomte du Nouy finds that the first rapid diminution of surface tension by adsorption in the surface layer is followed by a slower one, which may continue for some hours. An interesting fact is that the addition of a small quantity of sodium oleate or bile salt has only a temporary effect in lowering the surface tension of serum. The large fall produced at first is rapidly

recovered from, the surface tension returning to normal in a few minutes. This is held to be due to adsorption by the protein particles of the soap or bile salt, which are held in this way from exerting their activity elsewhere than on the surface of the particles. The fact is of importance as regards the absence of hæmolytic action by small amounts of bile salt in the circulating blood.

If it be wished to determine the dynamic surface tension, the wave method in the form devised by Hart-ridge and Peters (*Proc. Royal Society, A.*, vol. 101, p. 348) may be recommended.

It appears that certain changes may take place in blood in pathological states by which the surface tension is altered. Much further investigation is required in order to interpret the meaning of these changes.

It is possible that future investigation may also enable information to be obtained from measurements of surface tension as to the amount of substance in true solution and of the degree of aggregation of colloids. But the theoretical basis is not as yet sufficiently clear.

CHAPTER VII

IMBIBITION

THE fact that the dispersed phase of emulsoid colloids has the property of taking up more or less water has been referred to in the preceding pages. This property is fundamentally the same as that more commonly known as "imbibition," in which colloidal matter in a more or less solid or rigid condition absorbs water. This state is known as that of a "gel" or "jelly."

There is some confusion in the way these two terms are used. "Gel" may mean the coagulated or precipitated form of any colloid, suspensoid or emulsoid, whatever this form may be. Or, on the other hand, it may be used to refer to the state, also known as "jelly," to which a solution of gelatin changes when cooled. This latter state is characterized by its capacity of maintaining the shape into which it is cast, so that it approximates to a solid, although containing large quantities of water. It is also somewhat elastic, since after being slightly deformed by local pressure, a block of jelly returns to its original form when released. It seems advisable that a distinctive name should be given to this state, and since that of "gel" has become somewhat ill-defined, it may be suggested that "jelly" should be used, although this name may have to be extended to include substances like the stalk of *Laminaria*, which are not exactly jellies in the ordinary sense.

Nature of Jellies

What then is the nature of these "jellies"? It is clear that there must be some kind of structure present to enable them to keep their form. Opinions differ as to the kind of structure. There are various facts which show that there are two phases present, apart from those of the heterogeneous system of the colloid itself. One of these, the more solid one, containing less water than the other, forms some sort of framework. In the meshes of this framework there is a liquid phase, a more dilute colloidal solution. It seems probable that there are several different kinds of structure, and the following experiment by Hardy is instructive. If a solution of gelatin in water, say of 5 to 10 per cent. concentration, be allowed to cool, it forms the usual jelly. Although there are many reasons for believing that this system contains a liquid phase in its meshes, a very great pressure is required to press it out. If, however, a lump of the jelly is immersed in dilute formaldehyde, a change takes place such that the liquid can now be pressed out by squeezing with the hand. The most obvious explanation of the difference is that, in the first case, it is the solid phase that is continuous, enclosing drops of the liquid phase, as in the cells of a honeycomb. Thus, to be pressed out, these drops must be filtered through the solid. In the second case, the solid may be supposed to be in the form of a network like a sponge, consisting of a system of fibres connected together but with gaps through which the liquid phase can escape. Thus, both phases are here continuous ones, but interlaced together.

McBain's study of soaps leads him to the view that the structure is composed of particles or micelles, stuck together and probably arranged in the form of fibres, innumerable in number, with the ordinary

colloidal solution between them. He points out that the identity of the significant properties of sol and gel, such as electrical conductivity and osmotic pressure, indicates that there cannot be any great physical difference between them, at all events as a matter of necessity and in the case of soaps. "The process of gelatinization is such a subtle one that the colloidal particles are not sufficiently removed from the solution as to disturb the equilibrium existing in the latter" (*First Report of Adhesives Comm.*, Dept. Sci. and Indust. Research, p. 128). The view, taken by many, that the process is one of crystallization is incompatible with the evidence derived from soap solutions, if we regard crystallization to be the removal of substances from solution. On the other hand, soaps may pass into the form of a white curd, in which fibres can be seen by the ultra-microscope. This is the relatively insoluble form, and may possibly be crystalline.

Cause of Swelling

The nature of the forces causing swelling is obscure. Various theories have been put forward. Thus, Procter and Loeb regard the process as being essentially osmotic in origin. If the solid phase consists of a salt of gelatin, it may be looked upon as analogous to a colloidal salt enclosed in a parchment paper membrane as described below. The inorganic ions will be held by electrostatic attraction within the gelatin phase, since the latter is insoluble and unable to diffuse into the water. Thus, an osmotic pressure is created, which results in the attraction of water. The effect of the addition of neutral salts can also be accounted for by the operation of Donnan's membrane equilibrium if the above view is correct.

The precise situation into which the water is taken is

not very clear. If the closed cells of a honeycomb structure be present, the water may pass into their cavities and stretch their walls. But if the structure be similar to that of McBain's soaps, it appears that the water must either pass into the micelle and between the constituent molecules, driving them farther apart, or be adsorbed on the surface of the particle.

There seems to be evidence that the structural units of gelatin are capable of taking up water by adsorption on their surfaces. Indeed, Posnyak, in his investigations of the swelling of india-rubber and of gelatin, comes to the conclusion that there is taking up of water by "solid solution" in the particles in addition to adsorption on their surfaces. But the formula given suggests that adsorption is the chief factor. In a recent paper, Kuhn finds that the swelling of gelatin with organic acids follows an adsorption formula. But objection has been made that he did not take account of the hydrogen-ion concentration. However this may be, if the swelling in relation to the increase of hydrogen-ion concentration follows an adsorption law, rather than one of mass action, surface condensation is suggested.

Action of Salts

What has already been said suffices to show that in the emulsoid colloids we have to do with very complex systems, and that the interaction of the various factors involved is by no means elucidated. But we meet with yet a further complication in the action of neutral salts, as stated in the ionic series known by the name of Hofmeister.

The view put forward by Freundlich and by Hatschek, that the distribution of water between the two phases of an emulsoid system can be changed by various

agencies, has been already referred to. Whether this is the same problem as that of imbibition by solids is not quite clear, but the two are so closely related that they may be conveniently discussed together, since it appears to be most probable that the effects of salts in this respect are due to their action on certain properties of water and are similar in the two cases.

When a substance like *Laminaria* or gelatin imbibes water, it is found that the total volume of the system, solid plus water, is somewhat less than that of the sum of the two separate constituents, although, of course, the volume of the solid has greatly increased at the expense of the liquid phase. Hatschek refers to an experiment in which the compression of water when taken up by gum tragacanth was such that a pressure of 400 atmospheres would be necessary.

That molecular forces of sufficient magnitude to effect such compression of water are available is shown by the calculation of A. M. Williams, that the attractive (cohesion) pressure in the surface film on charcoal is about 10,000 atmospheres. He shows also that such compressive forces by their effect on electrolytic dissociation may give rise to a higher concentration of the ions of water in adsorbed layers, and a diffusion potential of the order of that observed in the case of suspensoids.

Incidentally it may be noted that heat is liberated in the process of imbibition of water by emulsoids, and that of the swelling of some gels has been measured. We may remember that heat is also evolved in the adsorption of gases by charcoal and in other adsorption processes.

We may take it then that water is compressed in the course of imbibition. Now, if we take the series of salts with different anions comprised in the Hofmeister series, we find that their relative effect on swelling is

in the order of their action on certain properties of water, expressed in compressibility, surface tension, and so on. We find a similar state of affairs in the dispersion of colloids such as globulin, as shown by Schryver, as also in the displacement of the temperature at which a phenol-water system becomes homogeneous (the "critical solution temperature"). Since, further, there are reasons for regarding the adsorption of ions as varying in degree in different cases, it is probable that this phenomenon plays a part in producing the changes in properties of water at the interface.

Acids and alkalies have a powerful effect in increasing the degree of swelling of colloids, since they cause an increase in the osmotically active protein salt. A theory of œdema on the basis of increased acidity of the blood has been put forward by Martin Fischer. Apart from the incapacity of such small changes in hydrogen-ion concentration as are possible in the blood to produce more than minute degrees of swelling in the presence of salts, there are other grounds, such as the existence of free liquid in the tissue spaces, which show that œdema is really a phenomenon of osmosis as explained above.

In the case of proteins, it is stated by Jacques Loeb that if the hydrogen-ion concentration is made the same in all cases, there is no difference between the effects of univalent ions; in other words, the Hofmeister series disappears. In view of the great difference met with between the action of individual ions, it is difficult to accept this view. It may be that higher concentrations than those for chemical combination with the protein in equivalent proportions are required to produce the results. But it is desirable that the experiments of Hofmeister and others should be repeated with equal hydrogen-ion concentrations in all cases. A few experiments that I have made myself

indicate that the series exists, even under the conditions referred to.

The swelling produced by acid is diminished by the presence of neutral salts. Loeb has shown that if we accept the osmotic origin of swelling, there would be a different concentration of the salt inside and outside the protein particles, which are assumed to be permeated through by the solution. There would be, in fact, a condition similar to Donnan's membrane equilibrium. The effective osmotic pressure of the protein would be decreased, and with this the amount of water taken up.

CHAPTER VIII

PHYSIOLOGICAL ACTION

IN the discussion of possible physiological or pharmacological action of colloids as distinct from those of the same substance in the molecular or ionic state, there are certain considerations to be kept in mind, especially as to the properties characteristic of the colloidal state.

In the first place, the purely chemical behaviour of those aggregations of molecules known as colloidal particles must clearly be a very inert one compared with that of the same amount of material in true solution. In the latter case, all the molecules are accessible to or can react with other molecules in the solution, provided there is some chemical interaction possible. Whereas, in the former, it is only those on the surface that can so react. In rare cases, however, it seems possible that spaces between the molecules of an aggregate may exist, so that there may be more or less free access of the liquid phase to all of the molecules of a particle. As already remarked, this is the view taken by Loeb as regards proteins. When only the surface is accessible, the product of reaction with an outer substance may be soluble and pass away into the liquid phase. The next layer then comes into action, and so on. If the product is insoluble, the surface becomes covered with it and reaction ceases. In either case, the effect is comparatively slow, so far as chemical behaviour is concerned.

Further, it is important to remember that a colloidal solution consists of a suspension of a more or less solid phase, itself containing water in the emulsoids, in a dilute true solution of the substance of which the particles are composed. While this true solution which constitutes the continuous phase may be extraordinarily dilute, as in silver chloride, barium sulphate or the proteins, in other cases, as in that of iodine or the free bases of some alkaloids, it attains a notable degree of concentration. The question naturally arises as to whether the action of colloidal preparations in general may not be entirely due to that part in true solution. Even when this is small, we have frequently to do with very powerful substances. Moreover, the considerations of the preceding paragraph may have an application, especially in the case of colloidal metals. If there be present in the blood or tissues some substance which reacts slowly with the surface of the particles, say of colloidal silver, there may be a continuous supply of a soluble silver salt in very small quantities as long as the particles remain. It is unnecessary to mention that such a process is just what is often required in the therapeutic use of powerful drugs. In many cases of commercial preparations, indeed, it has been found that the activity is equivalent to that of the fraction present in true solution. It often happens that this amount is greater than corresponds to the real solubility of the colloidal matter itself, and is due to the presence of a soluble salt of the substance in question. Thus in colloidal ferric hydroxide, there is nearly always a varying quantity of unchanged ferric chloride, which sometimes reaches an important concentration. The facts mentioned above have been established mainly by the work of Dale and of A. J. Clark on commercial preparations.

It may even be that organic compounds of metals,

such as salvarsan, owe their properties to slow decomposition with the giving off of the metal in ionized form. It should be remembered that the characteristic activities of a metal are only partially shown when it is in organic combination, and the view of Ehrlich, that these organic compounds have some special chemical affinity for parasitic organisms, seems to be losing its probability.

It seems, then, that the real value, if not the only one, of colloidal preparations of metals and other powerful drugs is to afford the very slow and prolonged action of continuous minute doses of the active ionized form. This, of course, may be of much practical utility.

A further difficulty in judging of the activity of such preparations is the fact that, in order to make them stable, various protectives are added. Even those colloids made by electrical disintegration of metallic electrodes, although they may be free from soluble salts of the metal, frequently contain some protective. The nature of this protective is not usually stated, and since it may be one with a powerful physiological action of its own, such as peptone or proteose, effects produced by intravenous injection cannot be ascribed to the colloid unless those of the suspension medium itself are known. Certain effects supposed to be due to colloidal gold have in point of fact been found to have been due to the protective (see especially the work of Auld).

It is not intended to deny the possibility of the electric charge on colloids being of some importance. But, apart from the fact that the actual amount of such substances introduced into the blood stream is so small as to make it difficult to accept the view that their charge plays any part in comparison with the universal occurrence of electric charges in the organism, the present writer has not met with any properly

controlled experiments which give proof of the activity of the electric charge as such. Of course, if a positively charged colloid, such as ferric hydroxide, is injected directly into the veins, it will cause precipitation of the negative proteins of the blood. It is used in the laboratory to remove proteins from serum. A negatively charged colloid would not do this. Hence it is only safe to use the latter in this way. But the negative charges thus introduced merely add a minute fraction to those already present.

Similar remarks might be made regarding the minute amount of specific adsorption possible on the small quantity of colloid introduced.

Since all vital activities take place in a system containing colloids, it is clear that a complete discussion of the part they play in the living organism would embrace the greater part of physiology. We are mainly concerned in the present chapter with the action of colloidal preparations introduced into the body. Although the colloidal state is common to many constituents of the body fluids, it must not be supposed that substances in true solution are absent or of any less importance than those in the colloidal form. Much less is it correct to say, as has been done, that drugs must be colloidal before they exert their effects, or that there is any value whatever in their being "isomorphic" with the components of the body. Indeed, the various membranes here present are impermeable to substances in the colloidal state, although large particles can be taken up by many cells. It may at first appear strange that large grains can pass through a membrane whose structure does not allow smaller ones to pass. The explanation lies in the nature of the cell membrane as a film deposited by adsorption at the surface. Large grains enter by actually making holes by mechanical violence as it were, but, as they pass

into the cell, a new membrane forms behind them by condensation of certain constituents of the cell. The process is similar to what happens when a needle is dropped through a soap film without breaking it. Perhaps metals might be made to enter cells in this way, in the form of grains larger than colloidal particles.

Enzymes

Although, as remarked, space forbids consideration of all the colloidal phenomena in the body, there are certain of these which have a direct connection with the pharmacological action of colloidal drugs. It is now generally recognized that the enzymes which play so important a part in the speeding up of chemical reactions in living organisms are a particular class of heterogeneous catalysts. In other words, they are colloids, and the reactions which they accelerate occur on the surface of the dispersed phase. There are many interesting results of this behaviour which do not properly belong to the subject-matter of this monograph. It is interesting, however, to refer to one or two facts. In the first place, the effect of degree of dispersion on the activity of an enzyme. The greater the number of particles into which a given mass of agent is subdivided, the greater is the surface of activity. Aggregating agents in general, especially electrolytes, have a depressant effect; but occasionally the aggregating effect is replaced by the opposite dispersing one, with increased activity. Again, since the reacting substances are condensed on the surface by a process of adsorption, it will be obvious that if foreign substances also present are adsorbed at the same time, they must displace to a greater or less extent the appropriate substrate. This is the way some inhibiting agents, such as saponin, act. It seems

also that certain of the products of the reaction displace substrate in this way, and that a part of their retarding effect is thus to be explained. But the possibility of their affecting dispersion must also be kept in mind, as also the increase of the reverse reaction. The complexity of the factors and the difficulty of formulating a complete mathematical expression for the velocity of reaction will be realized.

When a simple heterogeneous reaction, such as that of dissolving a piece of zinc in sulphuric acid, takes place, its rate depends on how fast the acid molecules diffuse to the zinc, and how fast the zinc sulphate diffuses away. But when the solid phase is in the form of colloidal particles in constant Brownian movement, as in enzymes, diffusion plays a negligible part, because the agent is uniformly present, and the distance which the substrate molecules have to travel is very short. Here the rate of reaction is controlled by that of the chemical reaction itself. The fact that an enzyme reaction may follow a unimolecular law, or that it has the temperature coefficient of a chemical reaction, throws no light of significance in the mechanism of the process as a whole.

As regards Brownian movement, it is incorrect to say that it is *necessary* for catalytic action, as has been claimed for certain colloids supposed to have a catalytic effect. Many of the catalysts used in industrial processes are deposited on the surface of porous masses.

The part played by the colloidal state of the products in the synthetic action of enzymes may appropriately be alluded to here. When any reversible reaction is concerned, what an enzyme does is to bring about the natural equilibrium, or an equilibrium near to it, at an extraordinarily rapid rate. Hence, whether these agents hydrolyse or synthesize depends on the state of the system. Thus, if the amylase of the liver

acts on glycogen, it hydrolyses it to glucose; if it acts on glucose, glycogen is formed. But the natural equilibrium of this reaction is very near to the glucose state, so that if glycogen remained in solution, there would be very little produced. This substance, however, being insoluble and colloidal is removed from participation in the chemical equilibrium, more is formed to restore this equilibrium, and the process goes on rapidly. From what has been said above with regard to the chemical inertness of colloids, it will be clear that actual separation of the product as a deposit is not necessary.

Catalytic Action

It is true that many metallic colloids, such as platinum, manganese and iron hydroxides, etc., act as catalysts for oxidation. They cause guaiacum resin to turn blue, for example. But no proof has yet been afforded that they produce any detectable increase in oxidation when introduced into the blood or tissues of an animal. Owing to the small amounts that it is possible to give in this way, it does not seem reasonable to expect any perceptible effect of this kind. On the other hand, so far as the writer is aware, the question has not been properly investigated, and we must not be too hasty in denying the possibility.

Ascoli and his co-workers in 1906 described experiments in which colloidal preparations of silver, gold and platinum were found to increase the activity of autolytic tissue enzymes, and stated that intravenous injection of such colloids in the living animal thus raises the nitrogen metabolism. Bradley has made a careful examination of such effects, and finds that they are only present when the colloidal preparations are either themselves acid or give off acid by decomposition.

When neutral, their action is nil. The results described are to be explained entirely by the acidity of the preparations. Autolysis is extremely sensitive to slight increase of acidity, and Bradley ascribes the atrophy resulting from defective blood supply to an organ as due to the development of acid (carbonic, lactic, etc.), increased autolysis thereby, and diffusion away of the products.

Osmotic Pressure

The part played by this property of certain colloids in the formation of urine and in the maintenance of the volume of the blood has been discussed in a previous chapter. It is interesting to note here that the addition of a colloid such as gum arabic to a solution for intravenous injection is perhaps the clearest case, if not the only one, where the colloidal properties as such form the purpose of the therapeutic use.

“Anaphylactoid” Phenomena

When serum is left in contact with certain colloidal materials, such as agar, it develops toxic properties of a kind regarded by some observers as similar to those concerned in anaphylactic shock. Dale and Kellaway have shown that the “anaphylatoxins” produced in this way owe their action to the formation of complexes which maintain the foreign serum colloids finely dispersed. Their effect in the whole animal is not the same as that in true anaphylactic shock, they do not act on isolated plain muscle but only in the circulating blood.

True anaphylaxis, as distinct from immunity, depends on the distribution of an antibody, probably a precipitin, between the cells and the body fluids. In

anaphylaxis the antibody has become chiefly located inside the cells, leaving the surrounding plasma nearly free. In immunity, there is a large amount of antibody in the circulation, so that when the antigen is injected, it is precipitated or neutralized before it reaches the cells. Whereas in anaphylactic shock the antigen is able to reach the cells without being destroyed. It reacts therein with the antibody present and in immediate relation with important structures. The problem as a whole is one of much difficulty, and the reader is referred to the work of Dale and Kellaway (*Phil. Trans.*, 1922).

CHAPTER IX

PROTEINS AND HÆMOGLOBIN

OWING to the special physiological importance of proteins, and particularly of that one known as hæmoglobin, it is desirable to devote some consideration to them in addition to the incidental references of earlier chapters.

As Amphoteric Electrolytes

Proteins, as containing both carboxyl and amino-groups in various numbers and proportions, act as bases in the presence of acids and as acids in the presence of inorganic bases. Thus, we may have a gelatin chloride or a sodium gelatinate, which will both be electrolytically dissociated, in the first case into chlorine anion and gelatin cation, in the second into sodium cation and gelatin anion. At a certain value of hydrogen-ion concentration, dependent on the relative numbers and strengths of the acidic and basic groups, a protein is not dissociated either as acid or base and therefore combines with neither. This is the so-called "isoelectric point," about which more will be said presently.

The ions of the electrolytically dissociated salts of a protein naturally have properties of viscosity, osmotic pressure, and so on which differ from those of the undissociated neutral substance without any inorganic

ions. It is contended by Loeb that all the properties of proteins can be accounted for on this view, which he considers as that of "classical chemistry" as opposed to that of the "colloid chemists." Apparently these latter are supposed to deny the chemical combination of proteins with acids and alkalies, and to explain all the phenomena by adsorption of hydrogen- or hydroxyl-ions. But there are surely very few colloid chemists who take this attitude. The formation of salts with acid and alkali has been familiar since the work of Bugarsky and Liebermann. Loeb seems to object to the introduction of the conceptions of aggregation and adsorption, while admitting all other physical considerations, such as membrane effects and electric charge. We have already found evidence of aggregation in the osmotic pressure of colloids, and if there is aggregation, there is the surface required for adsorption.

The work of Loeb has undoubtedly brought to light many important facts under the particular conditions of his experiments, but there are reasons for doubt as to whether all the properties of proteins can be explained by combination with acids or bases in equivalent proportion, apart from changes in physical state or adsorption. The intervention of the Donnan membrane equilibrium when other electrolytes are present is also thoroughly worked out and its importance emphasized. One would like, however, a more detailed investigation of the behaviour of uncombined protein in contact with pure water, where the Donnan membrane equilibrium does not intervene. Also of the osmotic and electrical properties of protein salts in absence of foreign salts or other electrolytes. Here also the Donnan membrane effect would not complicate matters.

It is perhaps well to state the reasons why adsorption

of ions alone does not give a satisfactory explanation of the effects of strong acids or bases on proteins. These reasons chiefly rest on the osmotic pressure. It was shown by Bugarsky and Liebermann that the depression of the freezing point of albumin solutions was much increased by the addition of hydrochloric acid or sodium hydroxide, not by the addition of sodium chloride. This implies a rise of osmotic pressure, and direct measurements in an osmometer have given the same result. Now it has been pointed out in Chapter IV. that the mere adsorption of an electrolyte on a colloidal particle cannot have the effect of increasing the osmotic pressure due to these particles, since it does not increase the total concentration of active elements and merely increases the mass of each of those already present. Moreover, there is sufficient evidence that the osmotic pressure which proteins show in acid or alkaline media is due chiefly or entirely to the inorganic ion, which can only be effective if kept within the membrane by the electrostatic attraction of the opposite indiffusible ion of an electrolytically dissociated salt, itself indiffusible. Thus, there is no doubt of the existence of such salts. But, it may be remarked incidentally, the protein ion is largely aggregated and must possess the properties of surface. If this be so, it is capable of taking up other substances by adsorption. By this means the concentration of these substances is lowered and important results may follow. Change in the degree of dispersion of proteins would also regulate the concentration of adsorbed substances in the external phase. It should further be remembered that it is difficult or impossible to attach much importance of a purely chemical nature to proteins in the blood plasma. They do not serve as nitrogen food to the cells, and are chemically inert compounds. We have seen the importance of their

osmotic pressure, and the process of clotting of the blood appears to be due to colloidal interaction.

The Isoelectric Point

We saw in Chapter III. that the electric charge on colloidal particles can be neutralized by the presence of ions of opposite sign. When deprived of charge, they no longer move in an electric field, and the concentration of a particular ion required to effect this change was called by Hardy the "isoelectric point." We see that the name refers to deprivation of charge by any ion. At the present time there is a tendency to restrict it to the hydrogen-ion, no doubt on account of the importance of this ion in the behaviour of amphoteric electrolytes, like the proteins. But this limitation of the meaning is misleading, and when intended to refer only to the hydrogen-ion, it should be so stated. There is a further risk in that when the sign of charge on any system can be reversed by the addition of acid or alkali, the conclusion is apt to be drawn that we have to do with an amphoteric electrolyte behaving in a simple chemical way. Two cases of this confusion may be noted by way of warning. The red blood corpuscles have been said to have an "isoelectric point," regardless of the fact that the origin of their charge lies in the nature of the membrane of the corpuscles as being permeable only to anions. The other case is that of cellulose, where the experiments of Perrin showed that one-twentieth normal hydrochloric acid did not completely abolish the charge. It is clear that this must be a question of adsorption, because if it were due to electrolytic dissociation of an amphoteric substance, it would imply very much stronger acid properties than cellulose actually possesses.

There are other reasons which excite doubts as to whether the whole behaviour of proteins is a mere question of salts with bases at a hydrogen-ion concentration below the isoelectric point, or with acids above this point. It is, for instance, rather difficult to explain on this simple view why the osmotic pressure rises so quickly to a high point as the alkalinity increases, but only slowly and not very high on the acid side, while it is scarcely possible to detect any change for some distance on both sides of this point. It appears, indeed, to be a region rather than a point. It is admittedly difficult to determine such small changes as those assumed in this region, although they should be there according to the theory of such systems. The very inert behaviour of proteins near the neutral reaction suggested to Gustav Mann the idea that in this region the acidic and basic groups are not free but combined together in a ring to form an internal salt of an ammonium type. Thus, the ring would require a certain concentration of acid or alkali in order to break it open and combine with either of these. This view is in accordance with certain experimental facts. One cannot detect any change in the reaction of a weak acid or base on the addition of serum proteins unless the hydrogen-ion concentration is above 10^{-4} or below about 10^{-9} . That of normal blood, although not so low as 10^{-9} , is below that of the isoelectric point of its proteins, and these should therefore be combined with sodium, whereas Cushny's ultra-filtration experiments showed that all the sodium was contained in the filtrate, while those of Neuhausen with a sodium electrode showed the sodium ions to be free from combination. It may be, of course, that the form of the curve about the isoelectric point rises so slowly on both sides that the combination is too small to be detected. But the result in practice is the same, and should make

us cautious in forming theories depending on small changes in reaction in the region of that of blood. Miss Jordan-Lloyd's results on the swelling of gelatin show that there is practically no difference along the region where it is minimal, *i.e.* between 10^{-4} and 10^{-9} . I found that there is no sodium caseinate formed by rubbing casein in a sodium phosphate solution of 10^{-7} or $10^{-7.5}$ in hydrogen-ion concentration, although this is considerably on the alkaline side of its isoelectric point. Whether there is combination or not at or near the reaction of the blood is clearly a point of physiological importance, and cannot be said as yet to be satisfactorily decided. Even if we do not admit the formation of ring compounds at and near the neutral and isoelectric points—it has not yet been disproved—the various published curves of the properties of proteins in relation to hydrogen-ion concentration show that there is very little protein salt at the reaction of the blood and tissues. It is frequently assumed that these constituents of the cell are in the form of “base-protein salts,” and theories of carriage of carbon dioxide, and to some extent acid-base equilibrium, have been built on this assumption. It is difficult to believe that so important a function as the removal of carbon dioxide should not be a more perfect mechanism.

Hæmoglobin

On account of the supreme importance of this protein, it is desirable to devote a few words to the way in which its colloidal properties take part in its behaviour.

Although it consists of a protein combined with another compound containing iron, it appears as a rule to be less aggregated than those of the serum,

since a membrane may be prepared which allows hæmoglobin to pass through, while holding back the serum proteins. At the same time, it is no doubt aggregated to a large extent, as shown by the fact that when dialysed against water its osmotic pressure is very low.

Its isoelectric point is said to be about $10^{-6.8}$, so that in presence of acids or bases at a hydrogen-ion concentration higher or lower than this, it should be combined with one or the other. But, on the other hand, it may well be that it is not so combined within the limits of about 10^{-4} and 10^{-9} . If this be so, it is not present in the blood as a sodium salt, and theories of the carriage of carbon dioxide and changes in acidity on combination with oxygen are wanting in basis. At any rate, the suggestions require experimental proof, and it is the chief object of the remarks made here to call attention to gaps in our knowledge. A large amount of detailed mathematical calculation has been made on what seems to be a rather uncertain experimental foundation.

Suppose, however, that hæmoglobin at the reaction of the blood ($10^{-7.5}$) is combined with sodium. Then unless precautions are taken to dialyse it against a solution of sufficient acidity, it follows that the substance, of which various measurements have been made, was really in part the sodium salt. This consideration applies especially to determinations of its osmotic pressure and of its power of taking up carbon dioxide. It is true that Bohr, with reference to his carbon dioxide dissociation curve, states that the hæmoglobin used was free from sodium, but the percentage weight of this element in the salt would be very small, and might conceivably escape detection by chemical analysis. If the sample of hæmoglobin used by Hüfner and Gansser for their determinations of its

osmotic pressure contained sodium salt, it is clear that deductions as to its molecular weight cannot safely be drawn from their figures.

It will be noticed that the isoelectric point of hæmoglobin is not far on the acid side of the neutrality of water. It must therefore behave as a very weak acid, in any case. The acidic dissociation constant, which gives its strength as an acid, can be calculated from the isoelectric point for hydrogen-ions. It comes out to be 2×10^{-8} . Now that of carbonic acid (for dissociation into HCO'_3 and H^+ ions) is 41.5×10^{-8} . So that if the two acids, both in normal concentration, are competing for the possession of sodium, their share is in proportion to their acidities. Hence less than 5 per cent. of the available sodium goes to the hæmoglobin. The medium in which hæmoglobin exists in the corpuscles is stated to be more acid than the plasma. This would bring it still nearer to the isoelectric point of hæmoglobin, and reduce still further the proportion combined with sodium. Any change due to the very slight difference in acidity of oxy- and reduced hæmoglobin seems to be within the limits of the errors of the electrometric method, and too trivial to have any physiological importance. The carriage of carbon dioxide by displacement of sodium from its combination with hæmoglobin would appear to be a very ineffective method, but it must not be overlooked that the calculation given above rests on assumptions which may not be correct, especially as regards the isoelectric point and its relation to the acidic dissociation constant. There is no doubt, however, that the phenomena require more *experimental* investigation.

In doing this, however, the fact that hæmoglobin is a colloid and therefore has properties of surface, including those of adsorption, must not be left out of account. The possibility that adsorption is one at

least of the factors controlling the taking up of oxygen has not yet been disproved. The nature of methæmoglobin as a true chemical compound with oxygen might throw light on the problem by comparison of its properties with those of oxy-hæmoglobin.

The reader is doubtless aware of the great value of the experimental work of Barcroft and his school, together with that of Haldane, on the various properties of hæmoglobin. These results will stand as the necessary basis for further work, but the present writer would enter a plea for an independent investigation in which no attempt is made to apply formulæ deduced from simple mass action until some more fundamental knowledge is acquired. It has been pointed out that the fact that a particular process, under certain conditions, obeys the unimolecular formula of rate of reaction does not prove that it is a simple chemical reaction in a homogeneous system, as is sometimes assumed. Many processes which are not chemical at all obey a similar law, which after all merely states that the rate at which a change occurs depends on the amount of the material still left unchanged. It applies to the increase of money at compound interest. In a series of processes of different rates depending on one another, the rate actually measured is that of the slowest. In many cases this is a chemical reaction, and may obey the law of mass action in a simple form. But this fact does not give any information about the nature of the other essential parts of the complete process.

That we have still much to learn about hæmoglobin is shown by the extraordinary range of values obtained by Adolph and Henderson for the heat of "combination" between oxygen and hæmoglobin, notwithstanding that the method used was of great accuracy. This seems to be due to some obscure colloidal changes,

but is a matter of great difficulty. It may be remarked incidentally that the fact that the "combination" of hæmoglobin with oxygen is attended with the liberation of heat does not prove that it is chemical in nature. We have seen above that the adsorption of gases by charcoal and of water by emulsoids also gives off heat. Indeed, in the former case the values are of the same order of magnitude as those of oxygen and hæmoglobin.

One of the pressing needs is a method of making uniform preparations of hæmoglobin, so that the researches of different workers may be capable of comparison with one another. The more careful and accurate the work, the greater seem to be the discrepancies. Nevertheless, there appears to be an impression that everything can be satisfactorily explained by mass action formulæ, whereas it is a matter of pure hypothesis that hæmoglobin behaves like a simple inorganic substance in true solution.

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